

AD \_\_\_\_\_

GRANT NUMBER: DAMD17-95-1-5004

TITLE: Vth Pan American Symposium on Animal, Plant and  
Microbial Toxins

PRINCIPAL INVESTIGATOR: Charlotte L. Ownby

CONTRACTING ORGANIZATION: Oklahoma State University  
Stillwater, OK 74078-0266

REPORT DATE: 30 Dec 96

TYPE OF REPORT: Final Proceedings

PREPARED FOR: Commander  
U.S. Army Medical Research and Materiel Command  
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;  
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

19970919 078

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
<small>Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.</small>				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 30 Dec 96	3. REPORT TYPE AND DATES COVERED ( 1 Dec 94 Final Proceedings - 30 Nov 95)		
4. TITLE AND SUBTITLE Vth Pan American Symposium on Animal, Plant and Microbial Toxins		5. FUNDING NUMBERS DAMD17-95-1-5004		
6. AUTHOR(S) Charlotte L. Ownby				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Oklahoma State University Stillwater, OK 74078-0266		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, Maryland 21702-5012		10. SPONSORING/MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT  Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words) <p>This report covers recent work in the areas of snake, plant, microbial and arthropod toxins. Presentations on snake toxins include work done on neurotoxins such as B-bungarotoxin and k-neurotoxins, phospholipase A<sub>2</sub> myotoxins, anti-muscarinic toxins, metalloproteinases, fibrinolytic enzymes, cardiotoxins, and antihemorrhagic factors. Presentations on plant and microbial toxins include work done on ricin, <u>Clostridium perfringens</u> enterotoxin, cone snail peptides, sea anemone toxins, proteinase inhibitors and maitotoxin. Presentations on arthropod toxins included work on scorpion neurotoxins, K<sup>+</sup> channel-blocking peptides, lice and wasp proteins, stinging insect venom allergens and Australian funnel-web spider toxins. Techniques and methods employed in these projects ranged from classical biochemistry, pharmacology and pathology to state-of-the-art molecular biology including cloning, expression and mutation studies.</p> <p style="text-align: center;"><b>DTIC QUALITY INSPECTED 4</b></p>				
14. SUBJECT TERMS snake venoms and toxins, plant toxins, microbial toxins, arthropod venoms and toxins		15. NUMBER OF PAGES 246		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

## GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet optical scanning requirements.

**Block 1. Agency Use Only (Leave blank).**

**Block 2. Report Date.** Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

**Block 3. Type of Report and Dates Covered.** State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

**Block 4. Title and Subtitle.** A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

**Block 5. Funding Numbers.** To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit Accession No.

**Block 6. Author(s).** Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

**Block 7. Performing Organization Name(s) and Address(es).** Self-explanatory.

**Block 8. Performing Organization Report Number.** Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

**Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es).** Self-explanatory.

**Block 10. Sponsoring/Monitoring Agency Report Number.** (If known)

**Block 11. Supplementary Notes.** Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

**Block 12a. Distribution/Availability Statement.** Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

**DOD** - See DoDD 5230.24, "Distribution Statements on Technical Documents."

**DOE** - See authorities.

**NASA** - See Handbook NHB 2200.2.

**NTIS** - Leave blank.

**Block 12b. Distribution Code.**

**DOD** - Leave blank.

**DOE** - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

**NASA** - Leave blank.

**NTIS** - Leave blank.

**Block 13. Abstract.** Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

**Block 14. Subject Terms.** Keywords or phrases identifying major subjects in the report.

**Block 15. Number of Pages.** Enter the total number of pages.

**Block 16. Price Code.** Enter appropriate price code (*NTIS only*).

**Blocks 17. - 19. Security Classifications.** Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

**Block 20. Limitation of Abstract.** This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

**INTERNATIONAL SOCIETY ON  
TOXINOLOGY  
PAN AMERICAN SECTION**

**5TH PAN AMERICAN SYMPOSIUM ON  
ANIMAL, PLANT AND MICROBIAL  
TOXINS**

**30 July - 4 August, 1995  
Frederick, Maryland**

**PROGRAM**



## TABLE OF CONTENTS

Conference Center Map .....	1
Committees .....	2
Acknowledgements .....	5
Social Program .....	6
Accompanying Persons Program .....	7
General Information .....	9

### SCIENTIFIC PROGRAM

#### Podium Sessions:

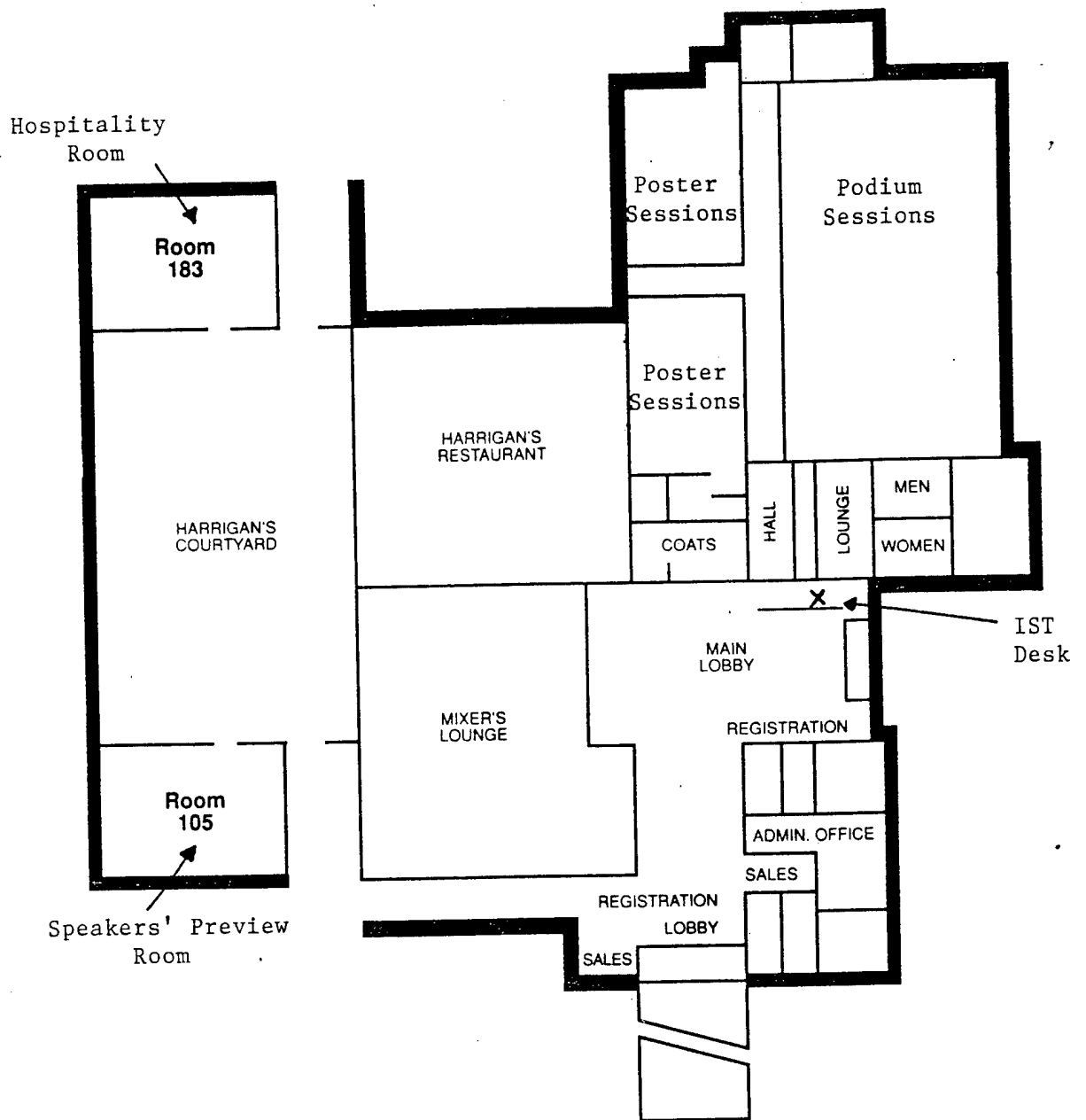
Monday, July 31 .....	11
Tuesday, August 1 .....	16
Wednesday, August 2 .....	19
Thursday, August 3 .....	21
Friday, August 4 .....	28

#### Poster Sessions:

Snake Toxins (1 - 50) .....	32
Bacterial Toxins (51 - 54) .....	40
Plant and Mycotoxins (55 - 62) .....	41
Marine Venoms (63 - 77) .....	43
Scorpion and Other Arthropods (78 - 82) .....	46
Marine and Aquatic Toxins (83 - 97) .....	47

### ABSTRACTS

Podium Sessions .....	50
Poster Sessions .....	131
AUTHOR INDEX .....	228



**Holiday Inn at FSK Mall - Conference Center**

## **ORGANIZING COMMITTEE**

**Chairperson: Kay A. Mereish**  
**Scientific Secretariat: Robert W. Wannemacher**

**David Franz**  
**Leonard Smith**  
**Bradley Stiles**  
**Richard Dinterman**  
**Charlotte Ownby**  
**Joseph W. Burnett**  
**Davy Jones**  
**Frederick W. Oehme**  
**John Hewetson**  
**Mark Poli**  
**Julia Franceschi**  
**Judy Pace**  
**Lourival Possani**  
**Donald Creasia**  
**Charles Templeton**  
**Frank Sexton**

## SESSION CHAIRS

### SNAKE VENOMS:

Leonard Smith  
Phil Rosenberg  
Charlotte Ownby  
Franc Gubensek  
Vincent Chiappinelli  
Andre Menez  
Jay Fox  
Jose Gutierrez  
Paul Reid  
M. Kini  
L. Rodriguez-Simioni

### BACTERIAL TOXINS:

Bradley Stiles  
Mary A. Woody  
Anna Johnson-Winegar  
Steve H. Leppla  
John Hewetson

### PLANT & MYCOTOXINS:

Frederick W. Oehme  
Kay A. Mereish  
Robert W. Wannemacher  
M. Ahmad

### MARINE VENOMS:

Joseph W. Burnett  
William Kem  
C. Alvarez  
J.C. Freitas

**SCORPIONS AND OTHER ARTHROPODS:**

Davy Jones  
T.P. King  
Lourival Possani  
P. Gopalakrishnakone  
C. Garcia  
Donald Creasia

**MARINE AND AQUATIC TOXINS:**

Mark Poli  
Dan Baden  
G. Escalona de Motta  
M.J. Holmes  
Olga Castaneda  
R. Frangez

## **ACKNOWLEDGMENTS**

**THE SCIENTIFIC COMMITTEE GRATEFULLY ACKNOWLEDGES THE  
SUPPORT AND SPONSORSHIP OF THE FOLLOWING:**

**BECKMAN INSTRUMENTS, INCORPORATED**

**MIAMI VALLEY SERPENTARIUM COMPANY**

**SIGMA XI, FREDERICK CLUB**

**CHAPMAN & HALL**

**U.S. ARMY**

**U.S. ARMY MEDICAL AND MATERIAL COMMAND**

**U.S. ARMY MEDICAL RESEARCH INSTITUTE AND INFECTIOUS DISEASES**

## **SYMPOSIUM SOCIAL PROGRAM**

**Please Bring Your Ticket to the Function and Wear Your Symposium Badge.**

**Sunday, 30 July 1995:**

**2000 -2200 Reception at Holiday Inn at Francis Scott Key Mall (No cost)**

**Monday, 31 July 1995: Free Time**

**Tuesday, 1 August 1995:**

**1800 - 2200 Crab feast - bus transportation to and from the crab feast (No cost).**

**Wednesday, 2 August 1995:**

**1200 - 1700 Lunch & tour at Gettysburg - bus transportation to Gettysburg National Park and return to Frederick; lunch at Cozy Restaurant in Thurmont. Restaurant; briefing with the electronic map at the visitors center; guided tour of the battlefield (No cost).**

**Thursday, August 3 1995:**

**1530 - 1730 Tour of USAMRIID (No Cost).**

**2000 - 2330 Farewell dinner at Holiday Inn, FSK Mall, Frederick (No cost).**

## **ACCOMPANYING PERSONS PROGRAM**

### **Monday, 31 July 1995:**

**0800 - 1000** Hospitality room open (Room 183).

**1000** Departure from Hotel.

**1015 - 1500** Walking tour of Historic Frederick, with guides; Carriage rides through Frederick; lunch (optional) at Tauraso's Restaurant; visit Frederick Museum & Information Center, Eveready Square, Shab Row, Carroll Creek Area, and Delaplaine Art Center (Cost only for lunch).

**1500** Departure from Historic Frederick to Hotel.

### **Tuesday, 1 August 1995:**

**0830 - 1000** Hospitality room open (Room 183).

**1000** Departure from Hotel.

**1030 - 1500** Tour of New Market (antique center of Maryland) - bus transportation to New Market and return to Frederick; tour of antique shops; lunch (optional) at Mealey's Inn (Cost only for lunch).

**1500** Departure from New Market.

**1800 - 2200** Crab feast (No cost).



**Wednesday, 2 August 1995:**

**9000 - 1000 Hospitality room open (Room 183).**

**1000 Departure from Hotel**

**1010 - 1200 Shopping tour at FSK Mall (No cost).**

**1200 Departure from Mall**

**1230 - 1700 Lunch & tour at Gettysburg (No cost), see page 6 for details.**

**Thursday, 3 August 1995:**

**0830 - 0900 Hospitality room open.**

**0900 Departure from Hotel**

**0945- 1300 Tour to Harper's Ferry - bus transportation to Harper's Ferry and Return to Frederick; tour of Harper's Ferry Wax Museum and National Park (No cost).**

**1300 - 1400 Lunch at Cliff Side Inn (optional, Cost only for lunch).**

**1400 Departure of Cliff Side Inn.**

**2000 - 2330 Farewell Dinner at Holiday Inn, FSK Mall, Frederick (No cost).**

**Friday, 4 August 1995:**

**0800 - 1200 Hospitality room open.**

## **GENERAL INFORMATION**

### **LOCATION:**

The conference will be held at the Holiday Inn at the Francis Scott Key Mall, I-270 at Route 85, Frederick, Maryland.

### **LANGUAGE:**

The official language for the meeting is English.

### **REGISTRATION DESK**

Sunday, 30 July	4:00 p.m. - 8:00 p.m.
Monday, 31 July	7:30 a.m. - 5:00 p.m.
Tuesday, 1 August	8:00 a.m. - 4:00 p.m.
Wednesday, 2 August	8:00 a.m. - 12:30 p.m.
Thursday, 3 August	8:00 a.m. - 4:00 p.m.
Friday, 4 August	8:00 a.m. - 2:00 p.m.

**BADGES :** PLEASE WEAR YOUR SYMPOSIUM BADGE DURING ALL FUNCTIONS. Lost badges will be replaced at a cost of \$30.00.

**ABSTRACT BOOK:** Lost program and abstract book can be replaced for \$20.00. Additional copy of abstract book can be purchased for \$20.00.

### **GROUND TRANSPORTATION**

#### **Shuttle Bus**

Participants that they need to be assisted in transportation from the hotel to the airport need to fill the "Shuttle Bus Service" form at the registration desk as soon as possible. Otherwise you may arrange for your own transportation by calling (Henry's Limo Service to BWI or to Washington-Dulles at 663-6310; or Brent Delivery Service to BWI; Washington-Dulles, Washington-National at 694-9383). The cost of transportation from the hotel to BWI or to Washington National is approximately \$70.00 per ride and from the hotel to Washington-Dulles is approximately \$60.00 per ride. The fare can be divided between 2-3 passengers.

**Taxi In Frederick:**

For local transportation or if you wish to make your own arrangements for taxi pick from hotel to airport, you may wish to call any of the following companies:

Taxi cab service at 301-662-2250.

Bowie transportation 301-695-0333

**Car Rental**

You may wish to make your own arrangement for car rental by contacting car rental companies or the official travel agency listed below.

**Official Travel Agency:**

**Carlson Travel Network**

**Travel Consultants**

**921 West Seventh Street, College Park Plaza**

**Frederick, Maryland 12701**

**Phone: 301-694-7575**

**1-800-843-0259**

**FAX: 301-694-3714**

The travel agency will be glad to help you with your airline ticket and car rental.

**PRE- AND POST- SYMPOSIUM TOURS WERE CANCELLED DUE TO INSUFFICIENT RESPONSE.**

**MONDAY, JULY 31, 1995**

**08:00 AM**                    **OPENING REMARKS**  
**Dr. Kay Mereish**  
**Dr. David Franz**  
United States Army Medical Research Institute of Infectious  
Diseases, Frederick, Maryland  
**Dr. Charlotte Ownby**  
President, International Society on Toxinology

**Session I: SNAKE TOXINS**

**Chairpersons : Dr. P. Rosenberg and Dr. Franc Gubensek**

**08:10 AM**                    **DOUBLE ROLE OF THE CHAPERON SUBUNIT OF CROTOXIN.** C. Bon\*, V. Choumet, E. Delot, G. Faure, A. Robbe-Vincent and B. Saliou. Unite des Venins, Institut Pasteur, Paris, France.

**08:30 AM**                    **MECHANISM OF ACTION OF  $\beta$ -BUNGAROTOXIN ( $\beta$ -BUTX), A PRESYNAPTICALLY ACTING PHOSPHOLIPASE A<sub>2</sub> (PLA<sub>2</sub>) NEUROTOXIN.** P. Rosenberg\*, E. Ueno and R. Chapell. Department of Pharmaceutical Sciences, University of Connecticut, School of Pharmacy, Storrs, CT. USA.

**08:50 AM**                    **SUBUNIT ASSOCIATIONS IN RATTLESNAKE PRESYNAPTIC NEUROTOXINS AND *PSEUDOCERASTES FIELDI* NEUROTOXIN.** Ivan I. Kaiser\*, Brian Francis, Jason Meng and Corrine Seebart. Department of Molecular Biology, University of Wyoming, Laramie, WY. USA.

**09:10 AM**                    **AFFINITY PURIFICATION AND CLONING OF TAIPOXIN-BINDING PROTEINS.** Anne K. Schlimgen, D. Dodds, S.-Y. Lu, J.A. Helms and M.S. Perin\*. Division of Neuroscience, Baylor College of Medicine, Houston, TX. USA.

- 09:30 AM      **MOLECULAR EVOLUTION OF PHOSPHOLIPASE A<sub>2</sub> TOXINS IN VIPERA AMMODYTES.** F. Gubensek\* and D. Kordis.  
Department of Biochemistry and Molecular Biology, J. Stefan Institute, Ljubljana, Slovenia.
- 09:50 AM      **ACCELERATED EVOLUTION OF SNAKE VENOM PHOSPHOLIPASE A<sub>2</sub> ISOZYMES TO ACQUIRE DIVERSE FUNCTIONS.** Tomohisa Ogawa\*<sup>1</sup>, Kin-ichi Nakashima<sup>1</sup>, Ikuo Nobuhisa<sup>1</sup>, Masanobu Deshimaru<sup>1</sup>, Yasuyuki Shimohigashi<sup>1</sup>, Yasuyuki Fukumaki<sup>2</sup>, Yoshiyuki Sakaki<sup>3</sup>, Shosaku Hattori<sup>3</sup> and Motonori Ohno<sup>1</sup>. <sup>1</sup>Department of Chemistry, Faculty of Science, and <sup>2</sup>Institute of Genetic Information, Kyushu University, Fukuoka 8<sup>12</sup> Institute of Medical Science, <sup>3</sup>The University of Tokyo, Tokyo, Japan.
- 10:10 AM      **COFFEE BREAK**

## Session II: SNAKE TOXINS

Chairpersons: Dr. Leonard Smith and Dr. Jay Fox

- 10:30 AM      **DO WE KNOW THE COMPLETE SEQUENCE OF METALLOPROTEINASE AND NONENZYMATIC PLATELET AGGREGATION INHIBITOR (DISINTEGRIN) PRECURSOR PROTEINS?** R. Manjunatha Kini. Bioscience Centre, Faculty of Science, National University of Singapore, Singapore.
- 10:50 AM      **SNAKE VENOM METALLOPROTEINASES: STRUCTURE, FUNCTION AND RELATIONSHIP TO THE MAMMALIAN ADAMs FAMILY OF PROTEINS.** Jay W. Fox\*<sup>1</sup>, Li-Guo Jia<sup>1</sup>, Jon B. Bjarnason<sup>2</sup> and Ken-Ichi Shimokawa<sup>1</sup>. <sup>1</sup>University of Virginia Health Sciences Center, Charlottesville, VA and <sup>2</sup>University of Iceland, Reykjavik, Iceland.

- 11:10 AM      **CHARACTERIZATION OF THE MAJOR METALLOPROTEASE ISOLATED FROM THE VENOM OF CROTALUS VIRIDIS OREGANUS.** Stephen P. Mackessy. Department of Biological Sciences, University of Northern Colorado, Greeley, CO.
- 11:30 AM      **POTASSIUM CHANNEL BLOCKING ACTIONS OF DENDROTOXINS.** A. Harvey. Department of Physiology And Pharmacology, SIDR, University of Strathclyde, Glasgow, UK.
- 11:50 AM      **SITE-DIRECTED MUTANTS OF DENDROTOXIN K REVEAL THAT POSITIVELY CHARGED RESIDUES FROM TWO STRUCTURAL DOMAINS OF THE PROTEIN ARE IMPORTANT FOR K<sup>+</sup> CHANNEL INTERACTION.** P. Reid<sup>1</sup>, F. Fancheng Wang<sup>2</sup>, Mark Olson<sup>1</sup>, James Schmidt<sup>1</sup>, Dave Parcej<sup>2</sup>, Oliver Dolly<sup>2</sup>, and Leonard Smith\*<sup>1</sup>, <sup>1</sup>USAMRIID, Fort Detrick, Maryland, USA and <sup>2</sup>Dept of Biochemistry, Imperial College, London. UK.
- 12:10 PM      **LUNCH**  
1:10 PM      **REVIEW OF POSTERS**

### **Session III: SNAKE TOXINS**

**Chairpersons : Dr. Vincent Chiappinelli and Dr. Andre Menez**

- 2:00 PM      **RECOMBINANT  $\alpha$ -BUNGAROTOXIN CONSTRUCTS AND MUTANT ANALYSIS.** E. Hawrot\*, Q-L. Shi and J.A. Rosenthal. Department of Molecular Pharmacology and Biotechnology, Division of Biology and Medicine, Brown University, Providence, RI. USA.
- 2:20 PM      **ON THE RECOGNITION SITE OF AN ANIMAL TOXIN, AS STUDIED BY SITE- DIRECTED MUTAGENESIS.** Denis Servent, Valerie Winckler-Dietrich, Cecile Fromen, Frederic Ducancel, Jean-Claude Boulain and Andre Menez\*. Centre d Etudes de Saclay, Cedex, France.

- 2:40 PM      **THE BINDING SITE FOR K-NEUROTOXINS ON NEURONAL NICOTINIC RECEPTORS PROBED WITH SNAKE-VENOM-DERIVED AND RECOMBINANT TOXINS.** V.A. Chiappinelli<sup>\*1</sup>, W.R. Weaver<sup>1</sup>, B.M. Conti-Fine<sup>2</sup>, J.J. Fiordalisi<sup>3</sup> and G.A. Grant<sup>3</sup>. <sup>1</sup>St. Louis University, St. Louis, MO, <sup>2</sup>University of Minnesota, St. Paul, MN and <sup>3</sup>Washington University, St. Louis, MO. USA.
- 3:00 PM      **ANTI-MUSCARINIC TOXINS FROM *DENDROASPIS ANGUSTICEPS*.** L.T. Potter\*, J.M. Carsi-Gabrenas, S.L. Purkerson, M.P. Santiago, W.L. Strauss and H.H. Valentine. Department of Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL. USA.
- 3:20 PM      **COFFEE BREAK**

#### Session IV: SNAKE TOXINS

Chairpersons: Dr. Charlotte Ownby and Dr. Jose Gutierrez

- 3:35 PM      **ATROXASE: A FIBRINOLYTIC ENZYME ISOLATED FROM THE VENOM OF WESTERN DIAMONDBACK RATTLESNAKE: ISOLATION, CHARACTERIZATION AND CLONING.** Brenda J. Baker<sup>1</sup> and Anthony T. Tu<sup>\*2</sup>. <sup>1</sup>Department of Chemistry, University of Southern Colorado, Pueblo, CO and <sup>2</sup>Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO. USA
- 3:55 PM      **STUDIES ON THE MECHANISM OF ACTION OF MYOTOXINS ISOLATED FROM BOTHROPS SNAKE VENOMS.** J.M. Gutierrez\* and B. Lomonte. Instituto Clodomiro Picado, Facultad de Microbiologia, Universidad de Costa Rica, San Jose, Costa Rica.

- 4:15 PM            **SIMILARITIES AND DIFFERENCES IN MECHANISMS OF  
CARDIOTOXINS, MELITTIN AND OTHER MYOTOXINS.** J.E.  
Fletcher\*, Q-H. Gong and M-S. Jiang. Medical College of  
Pennsylvania and Hahnemann University, Philadelphia, PA. USA.
- 4:35 PM            **MYOTOXICITY OF VENOMS AND TOXINS.** P.  
Gopalakrishnakone. Venom and Toxin Research Group, National  
University of Singapore, Singapore.
- 4:50 PM            **cDNA CLONING AND PROTEIN SEQUENCE ANALYSIS OF A  
K49-PLA2 MYOTOXIN: A HIGHLY CONSERVED CLASS OF  
PROTEINS.** Heloisa S. Selistre de Araujo\*<sup>1,3</sup>, Steven P. White<sup>2</sup>  
and Charlotte L. Ownby<sup>1</sup>. <sup>1</sup>Department of Physiological Sciences  
and <sup>2</sup>Department of Biochemistry and Molecular Biology,  
Oklahoma State University, Stillwater, OK, and <sup>3</sup>Department de  
Ciencias Fisiologicas, Universidade Federal de Sao Carlos, Sao  
Carlos, Brazil.
- 5:05 PM            **PARTIAL BIOCHEMICAL CHARACTERIZATION OF A  
MYOTOXIN FROM THE DUVERNOY'S SECRETION OF  
PHILODRYAS OLFERSII.** J. Prado-Franceschi\*<sup>1</sup>, S. Hyslop<sup>1</sup>, J.C.  
Cogo<sup>1</sup>, A.L. Andrade<sup>1</sup>, A.P. Reichl<sup>3</sup>, M.T. Assakura<sup>3</sup>, M.A.  
Cruz-Hofling<sup>2</sup> and L. Rodrigues-Simioni<sup>1</sup>. Departments of  
<sup>1</sup>Pharmacology and <sup>2</sup>Histology, UNICAMP, Campinas and  
<sup>3</sup>Laboratory of Biochemistry and Biophysics, Instituto Butantan, Sao  
Paulo, Brazil.
- 5:15 PM            **ERINACIN, AN ANTIHAEMORRHAGIC FACTOR FROM THE  
EUROPEAN HEDGEHOG, ERINACEUS EUROPAEUS.** D.  
Mebs\*<sup>1</sup>, T. Omori-Sato<sup>2</sup>, Y. Yamakawa<sup>2</sup> and Y. Nagaoka<sup>2</sup>.  
<sup>1</sup>Zentrum der Rechtsmedizin, University of Frankfurt, Frankfurt,  
Germany and <sup>2</sup>National Institute of Health, Tokyo, Japan.



**TUESDAY, AUGUST 1, 1995**

**Session I: MICROBIAL TOXINS**

**Chairpersons: Dr. Mary Woody and Dr. Bradley Stiles**

- 08:30 AM      **BACTERIAL ENTEROTOXINS AFFECTING THE COLON.** Tracy D. Wilkins. Center for Biotechnology, Virginia Tech, Blacksburg, VA. USA.
- 09:05 AM      **OVERVIEW OF CLOSTRIDIUM PERFRINGENS ENTEROTOXIN.** Bruce A. McClane, University of Pittsburgh School of Medicine, Pittsburgh, PA. USA.
- 09:40 AM      **THE STRUCTURE AND MECHANISM OF ACTION OF BOTULINUM TOXIN.** Nabil Bakry, Julie Coffield, Andrew B. Maksymowych, Ren-de Zhang and Lance L. Simpson\*. Division of Environmental Medicine and Toxicology, Department of Medicine, Jefferson Medical College, Philadelphia, PA. USA.
- 10:15 AM      **TRANSMEMBRANE VOLTAGE AND pH GRADIENTS FACILITATE THE TRANSLOCATION OF TETANUS TOXIN THROUGH MEMBRANES DEVOID OF RECEPTORS.** H. Bigalke\*, T. Binscheck, F. Bartels. Institute of Toxicology, Medical School of Hannover, Hannover, Germany.
- 10:35 AM      **PURIFICATION AND STRUCTURE-FUNCTION STUDIES OF A HEMAGGLUTININ FROM TYPE A CLOSTRIDIUM BOTULINUM.** F.-N. Fu\*, S.K. Sharma and B.R. Singh. Department of Chemistry, University of Massachusetts Dartmouth, N. Dartmouth, MA. USA.
- 10:50 AM      **COFFEE BREAK**

## **Session II: MICROBIAL TOXINS**

**Chairpersons: Dr. Anna Johnson-Winegar, Dr. B. Stiles and Dr. M. Woody**

- 11:05 AM THE STAPHYLOCOCCAL ENTEROTOXINS.** Merlin S. Bergdoll.  
Food Research Institute, University of Wisconsin, Madison, WI.  
USA.
- 11:40 AM STRUCTURE-FUNCTION STUDY OF TOXIC SHOCK  
SYNDROME TOXIN-1 (TSST-1).** Peter Bonventre\*, Edmond Choi,  
Lorna Blanco, Holly Heeg and Constance Cullen. Department of  
Molecular Genetics, Biochemistry and Microbiology, University of  
Cincinnati Medical Center, Cincinnati, OH. USA.
- 12:15 AM INHIBITION OF STAPHYLOCOCCAL ENTEROTOXIN  
B(SEB)-STIMULATED MITOGENICITY IN HUMAN T-CELL  
CULTURES BY BLOCKING LIPOXYGENASE, BUT NOT  
CYCLOOXYGENASE PATHWAYS.** Marti Jett\*, Chris Welch,  
Sheila McMorro, Thomas Boyle, David Hoover and Peter  
Gemski. Walter Reed Army Institute Research, Washington, D.C.  
USA.
- 12:35 PM LUNCH & REVIEW OF POSTERS**

## **Session III: MICROBIAL TOXINS**

**Chairpersons: Dr. S. Leppla and B. Stiles**

- 1:45 PM BINDING AND UPTAKE OF ANTHRAX TOXIN COMPONENTS  
AND FUSION PROTEINS BY EUKARYOTIC CELLS.** S.H.  
Leppla\*, K.R. Klimpel, V.M. Gordon, N. Arora and Y. Singh.  
Laboratory of Microbial Ecology, National Institute of Dental  
Research, NIH, Bethesda, MD. USA.

2:20 PM            **SHIGA-LIKE TOXINS OF ENTEROHEMORRHAGIC E. coli.**  
Alison D. O'Brien, Department of Microbiology and Immunology,  
USUHS, Bethesda, MD. USA.

2:55 PM            **CHOLERA ENTEROTOXIN (CT) AND CT-LIKE**  
**ENTEROTOXIN.** Richard A. Finkelstein. School of Medicine,  
University of Missouri, Columbia , MO. USA.

3:30 PM            **COFFEE BREAK & POSTER REVIEW**

3:45 PM            **SYMPOSIUM PHOTO**

4:00 PM            **POSTER SESSION REVIEW**

5:00 PM            **BUSINESS MEETING**

6:30 PM            **CRAB FEAST**

**WEDNESDAY, AUGUST 2, 1995**

**Session I: PLANT TOXINS, MYCOTOXINS**

**Chairpersons: Dr. Frederick W. Oehme and Dr. Kay A. Mereish**

- 08:30 AM INTRODUCTION TO PLANT AND FUNGAL TOXINS.** Frederick W. Oehme, Kansas State University, Manhattan, KS. USA.
- 08:35 AM STRUCTURAL ASPECTS OF RICIN BEARING ON DRUG DESIGN AND MEMBRANE TRANSLOCATION.** Jon D. Robertus\*, Jorg Steighardt, Maria Svinth, Philip Day, Arthur Monzingo and Stephen Ernst. Department of Chemistry and Biochemistry, University of Texas, Austin, TX. USA.
- 08:55 AM THE CYTOTOXIC RIBONUCLEASE  $\alpha$ -SARCIN: THREE-DIMENSIONAL STRUCTURE; RIBOSOMAL RNA IDENTITY ELEMENTS FOR SPECIFIC RECOGNITION; AND AN ENGINEERED CHANGE IN THE RNA SUBSTRATE THAT AFFECTS ENZYME SPECIFICITY.** Ira G. Wool, Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL. USA.
- 09:15 AM NUCLEOSIDE N-RIBOHYDROLASE INHIBITOR DESIGN APPLIED TO RICIN A-CHAIN.** Todd Link, Xiangyang Chen, Lin-Hao Niu and Vern L. Schramm\*. Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY. USA.
- 09:35 AM RIBOSOME INACTIVATING ENZYMES IN CASTOR BEAN AND MAN.** Richard J. Youle. NINDS, National Institutes of Health, Bethesda, MD. USA.
- 10:00 AM COFFEE BREAK**

**WEDNESDAY, AUGUST 2, 1995**

**Session II: PLANT TOXINS, MYCOTOXINS**

**Chairpersons: Dr. Frederick W. Oehme and Dr. Robert Wannemacher**

- 10:20 AM      **PLANT TOXINS OVERVIEW.** Gerhard G. Habermehl. Department of Chemistry, School of Veterinary Medicine, Hannover, Germany.
- 10:40 AM      **TOXIGENIC FUNGI IN THE AIR WE BREATHE.** Bruce B. Jarvis. Department of Chemistry and Biochemistry, University of Maryland, College Park, MD. USA.
- 11:00 AM      **STRUCTURE-ACTIVITY RELATIONSHIPS FOR PHYTOTOXICITY AND MAMMALIAN CYTOTOXICITY OF THE SPHINGOSINE ANALOG MYCOTOXINS FUMONISIN B1 AND AAL-TOXIN.** W.T. Shier<sup>\*1</sup>, F.A. Badria<sup>1</sup> and H.K. Abbas<sup>2</sup>.  
<sup>1</sup>University of Minnesota, Minneapolis, MN and <sup>2</sup>Southern Weed Science Laboratory, USDA/ARS, Stoneville, MS. USA.
- 11:20 AM      **CYANOBACTERIAL BLOOMS AND HEALTH HAZARDS - AN OVERVIEW.** Wayne W. Carmichael. Department of Biological Sciences, Wright State University, Dayton, OH. USA.
- 11:40 AM      **QUESTIONS, DISCUSSION, SESSION SUMMARY**
- 12:30 PM      **GETTYSBURG TOUR AND LUNCH**

**THURSDAY, AUGUST 3, 1995**

**Session I: MARINE VENOMS**

**Chairpersons: Dr. Joseph Burnett and Dr. William Kem**

- 08:30 AM**            **RECENT ADVANCES IN COELENTERATE VENOM RESEARCH 1991-1995: CLINICAL, CHEMICAL AND IMMUNOLOGICAL ASPECTS.** Joseph W. Burnett, David Bloom, Shinichi Imafuku, C. Lisa Kauffman, Laure Aurelian, and Samuel Morris, Baltimore and Columbia, MD. USA.
- 09:00 AM**            **IDENTIFICATION OF ESSENTIAL RESIDUES IN THE POTASSIUM CHANNEL INHIBITOR ShK TOXIN: ANALYSIS OF MONOSUBSTITUTED ANALOGS.** William R. Kem\*, Michael W. Pennington<sup>1</sup>, Vladimir M. Mahnir, Michael E. Byrnes<sup>1</sup>, Inna Zaydenberg<sup>1</sup>, Ilya Khaytin<sup>1</sup>, Doug S. Krafte<sup>2</sup>, Roger Hill<sup>2</sup> and Jose de Chastonay<sup>1</sup>. Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL, <sup>1</sup>Bachem Bioscience Inc., King of Prussia, PA and <sup>2</sup>Sterling-Winthrop, Collegeville, PA. USA.
- 09:20 AM**            **CARDIOVASCULAR EFFECTS OF PHYSALIA VENOM.** David Hessinger. No Abstract.
- 09:40 AM**            **BIOLOGICAL AND BIOCHEMICAL STUDIES ON THE VENOM OF THE POISONOUS FISH THALASSOPHRYNE NATTERERI (NIQIUM).** M.L. Ferreira, K.C. Barbaro\*, D.F. Cardoso and I. Mota. Laboratory of Immunopathology-Butantan Institute-Sao Paulo, Brazil.
- 09:55 AM**            **MASS SPECTROMETRIC INVESTIGATIONS ON CONUS PEPTIDES.** T. Krishnamurthy\*, M. Prabhakaran. U.S. Army Edgewood RD & E Center, Aberdeen Proving Ground, MD. USA.
- 10:10**                **COFFEE BREAK**

**THURSDAY, AUGUST 3, 1995**

**Session II: MARINE VENOMS**

**Chairpersons: Dr. Joseph Burnett and Dr. C. Alvarez**

- 10:30 AM      **COMPARATIVE TOXINOLOGY OF VENEZUELAN SPONGES FROM THE GENUS HALICLONA.** C. Sevcik\*, M. Ramos and G. D'Suze. Instituto Venezolano de Investigaciones Cientificas, Centro de Biofisica y Bioquimica, Laboratory of Cellular Neuropharmacology, Caracas, Venezuela.
- 10:45 AM      **PATHOPHYSIOLOGY OF A NOVEL PROTEIN UpI FROM THE SEA ANEMONE URTICINA PISCIVORA.** E.I. Cline\*, Michael W. Wolowyk, D. Biggs and L.I. Wiebe. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada.
- 11:00 AM      **FLUORESCENT LABELED  $\alpha$ -CONOTOXIN GI: BINDING INTERACTIONS WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR AND MONOCLONAL ANTIBODIES.** J.D. Ashcom\* and B.G. Stiles. Toxinology Division, USAMRIID, Fort Detrick, Frederick, MD. USA.
- 11:15 AM      OPEN
- 11:30 AM      **NOVEL PRIMARY STRUCTURE OF STICHOLYSIN AND ITS INTERACTION WITH MEMBRANES.** C. Alvarez\*<sup>1</sup>, M. Tejuca<sup>1</sup>, V. Besada<sup>2</sup>, F. Pazos<sup>2</sup>, M.E. Lanio<sup>1</sup>, G. Padron<sup>2</sup>. <sup>1</sup>Universidad de La Habana and <sup>2</sup>Centro de Ingenieria Genetica y Biotecnologia, Havana, Cuba.

11:45 AM	<b>PURIFICATION, CHARACTERIZATION AND IMMOBILIZATION OF PROTEINASE INHIBITORS FROM STICHODACTYLA HELIANTHUS.</b> J. Delfin* <sup>1</sup> , J. Diaz <sup>1</sup> , W. Antuch <sup>2</sup> , R. Rodriguez <sup>2</sup> , Y. Gonzalez <sup>1</sup> , V. Morera <sup>2</sup> , J. Martinez <sup>1</sup> , N. Larionova <sup>3</sup> , G. Padron <sup>2</sup> and M. Chavez <sup>1</sup> . <sup>1</sup> Facultad de Biologia, Universidad de La Habana, <sup>2</sup> Centro de Ingenieria Genetica y Biotecnologia, La Habana, Cuba and <sup>3</sup> Faculty of Chemistry, Moscow State University, Russia.
12:00	<b>LUNCH &amp; REVIEW OF POSTERS</b>
3:30 PM	<b>USAMRIID TOUR</b>
8:15 PM	<b>FAREWELL DINNER AT THE HOLIDAY INN</b>



**THURSDAY, AUGUST 3, 1995**

**Session I: SCORPIONS AND OTHER ARTHROPODS  
STRUCTURE AND FUNCTION OF ARTHROPOD TOXINS**

**Chairpersons: Dr. Davy Jones and Dr. T.P. King**

- 08:30 AM      **INSECT VENOM ALLERGENS.** T.P. King. Rockefeller University, New York, NY. USA.
- 08:55 AM      **ANALYSIS OF THE STRUCTURE OF VENOMOUS PROTEINS FROM PARASITIC INSECTS.** Davy Jones. Graduate Center for Toxicology, University of Kentucky, Lexington, KY. USA.
- 09:20 AM      **ENDOCRINOLOGICALLY ACTIVE VENOM PROTEINS OF ECTOPARASITIC WASPS.** Thomas A. Coudron. USDA, ARS, BCIRL, Columbia, MO. USA.
- 09:45 AM      **PHARMACOLOGY OF SALIVA IN BLOOD-FEEDING HEMIPTERANS.** D. E. Champagne<sup>\*1</sup>, J.H. Valenzuela<sup>2</sup>, R. H. Nussenzweig<sup>2</sup>, and Jose M.C. Ribeiro<sup>2</sup>, <sup>1</sup>Department of Veterinary Science and Center for Insect Science, <sup>2</sup>Department of Entomology and Center for Insect Science, University of Arizona, Tucson, AZ. USA. No Abstract.
- 10:10          **COFFEE BREAK.**
- 10:20 AM      **IMMUNIZATION AGAINST A PEPTIDE VASODILATOR FROM SAND FLY SALIVA PROTECTS AGAINST LEISHMANIA TRANSMISSION IN MICE.** C.B. Shoemaker\*, A.A. Siddiqui, P.J. Skelly, G.C. Lanzaro, A.H. Santos, J.N. Ribeiro, and R.G. Titus, Department of Tropical Public Health, Harvard School of Public Health, Boston, MA. USA.

10:45 AM	<b>STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF NOXIUSTOXIN, A K<sup>+</sup> CHANNEL BLOCKING-PEPTIDE FROM THE VENOM OF THE SCORPION CENTRUROIDES NOXIUS.</b> L.D. Possani*, G.B. Gurrola, F. Martinez and B. Becerril. Department of Molecular Recognition and Structural Biology, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico.
11:10 AM	<b>BEE AND ANT VENOM PROTEINS.</b> Donald Hoffman, Dept of Pathology and Laboratory Medicine, East Carolina University, Greenville, NC, USA. No Abstract.
11:25 AM	<b>INSECTICIDAL TOXINS FROM THE SPIDERS <i>DIGUETIA CANITICS</i>, <i>TEGENARIA AGRESLIS</i>, AND <i>FILISTATA HIBERNALIS</i>: CHEMICAL CHARACTERIZATION, MOLECULAR BIOLOGY, BIOLOGICAL ACTIVITY, AND POTENTIAL USES IN THE DEVELOPMENT OF NEW INSECTICIDES.</b> Robert M. Kral, Jr.*, Karen J. Krapcho, Janice Johnson, Eric DelMar, Bradford C. VanWagenen, Irene V. Capuano, Christine K. Dunn, Forrest H. Fuller, Kathryn G. Eppler and Terry K. Morgan. NPS Pharmaceuticals, Inc., Salt Lake City, UT. USA.
11:40 AM	<b>OBSERVATIONS OF THE POTENTIAL COMMERCIAL USE OF ARTHROPOD VENOM MOLECULES.</b> R. Todd. No Abstract.
12:00 PM	<b>LUNCH</b>
1:00 PM	<b>REVIEW OF POSTER SESSION</b>
2:00 PM	<b>SYMPOSIUM ON SCORPION AND OTHER ARTHROPOD TOXINS-</b> Next room
3:30 PM	<b>USAMRIID TOUR</b>
8:15 PM	<b>FAREWELL DINNER AT HOLIDAY INN</b>

THURSDAY, AUGUST 3, 1995

Session II: SCORPIONS AND OTHER ARTHROPODS

Chairpersons: Dr. L. Possani and Dr. Gopalakrishnakone

- 2:00 PM             **$\alpha$ -SCORPION TOXINS BINDING ON RAT BRAIN AND INSECT SODIUM CHANNELS REVEAL DIVERGENT ALLOSTERIC MODULATIONS BY BREVETOXIN AND VERATRIDINE.**  
Sandrine Cestele<sup>1</sup>, Rym Ben Khalifa<sup>2</sup>, Marcel Pelhate<sup>2</sup>, Herve Rochat<sup>1</sup> and Dalia Gordon<sup>\*1</sup>. <sup>1</sup>Faculty of Medicine Nord, I.F.R. Jean Roche, Laboratory of Biochemistry, Marseille Cedex, France, and <sup>2</sup>Laboratory of Neurophysiology, University of Angers, Angers Cedex, France.
- 2:15 PM            **NON-ADRENERGIC NON-CHOLINERGIC (NANC) ACTION OF THE INDIAN RED SCORPION VENOM.** M.C.E. Gwee\*, L.S. Cheah and P. Gopalakrishnakone<sup>1</sup>. Venom and Toxin Research Group, Departments of Pharmacology and <sup>1</sup>Anatomy, Faculty of Medicine, National University of Singapore, Kent Ridge, Singapore.
- 2:30 PM            **ACUTE PHASE RESPONSE FOLLOWING SCORPION STING IN CHILDREN.** S. Sofer. Pediatric Intensive Care Unit, Soroka Medical Center and Ben-Gurion University of the Negev, Beer-Sheva, Israel.
- 2:45 PM            **MECHANISMS OF SECRETORY DISCHARGE STIMULATED BY SCORPION VENOM.** P.L. Fletcher\*, M.D. Fletcher, L.K. Fainter and S.M. Lucas. E.C.U. School of Medicine, Greenville, NC. USA.
- 3:00 PM            **ISOLATION AND CHARACTERIZATION OF THE GENES ENCODING GAMMA TOXIN OF THE BRAZILIAN SCORPIONS TITYUS SERRULATUS, TITYUS BAHIENSIS AND TITYUS STIGMURUS.** B. Becerril\*, M. Corona and L.D. Possani. Department of Molecular Recognition and Structural Biology, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico.

- 3:15 PM            **SELECTIVE MODIFICATION OF SODIUM CHANNEL  
INACTIVATION BY VERSUTOXIN AN AUSTRALIAN  
FUNNEL-WEB SPIDER TOXIN.** G.M. Nicholson\*<sup>1</sup> and T.  
Narahashi<sup>2</sup>. <sup>1</sup>Department of Biochemistry and Physiology,  
University of Technology, Sydney, Australia and <sup>2</sup>Department of  
Pharmacology, Northwestern University Medical School, Chicago,  
IL. USA.
- 3:30 PM            **TOUR OF USAMRIID**
- 8:15 PM            **FARWELL DINNER AT THE HOLIDAY INN**

FRIDAY, AUGUST 4, 1995

Session I: MARINE AQUATIC TOXINS

Chairperson: Dr. Mark Poli and D. Baden

- 08:00 AM      **CIGUATERA (FISH POISONING): PROGRESS AND PERSPECTIVES.** Richard J. Lewis. Queensland Department of Primary Industries, QABC, Gehrmann Laboratories, The University of Queensland, Australia.
- 08:50 AM      **ASSESSMENT OF METHODS FOR THE DETERMINATION OF CIGUATOXINS.** R. W. Dickey\*<sup>1</sup>, R. L. Manger<sup>2</sup>, M. A. Poli<sup>3</sup>, F. Van Dolah<sup>4</sup>, J. A. Delgado-Arias<sup>5</sup>, S. M. Musser<sup>6</sup>, D.G. Baden<sup>5</sup>, J. Hungerford<sup>2</sup>, K. S. Rein<sup>5</sup>, S. Lee<sup>2</sup>, T. Leighfield<sup>4</sup> and H. R. Granade<sup>1</sup>. <sup>1</sup>FDA, GCSL, Dauphin Island, AL, <sup>2</sup>FDA, SPRC, Bothell, WA, <sup>3</sup>USAMRIID, Fort Detrick, Frederick, MD, <sup>4</sup>NMFS, SFSC, Charleston, SC, <sup>5</sup>NIEHS, University of Miami, Miami, FL and <sup>6</sup>FDA, IBB, Washington, D.C. USA.
- 09:10 AM      **ANALYTICAL MEASUREMENT OF CIGUATOXIN LEVELS IN FISH TISSUE USING MASS SPECTROMETRY.** Steven M. Musser\*<sup>1</sup>, H. Ray Granade<sup>2</sup> and Robert W. Dickey<sup>2</sup>. <sup>1</sup>Center for Food Safety and Applied Nutrition, FDA, Washington, D.C. and <sup>2</sup>Gulf Coast Seafood Laboratory, FDA, Dauphin Island, AL. USA.
- 09:30 AM      **SUBMICROMOLE STRUCTURE ELUCIDATION USING MICRO INVERSE DETECTION--APPLICATIONS TO MARINE TOXIN STRUCTURE ELUCIDATION.** G.E. Martin\*<sup>1</sup>, R.C. Crouch<sup>1</sup>, R.W. Dickey<sup>2</sup>, H.R. Granade<sup>2</sup> and S.M. Musser<sup>3</sup>. <sup>1</sup>Bioanalytical Sciences NMR Laboratory, Burroughs Wellcome Co., Research Triangle Park, NC, <sup>2</sup>Gulf Coast Seafood Laboratory, USFDA, Dauphin Island, AL and <sup>3</sup>Instrumentation and Biophysics Branch, FDA, Center for Food Safety and Applied Nutrition, Washington, D.C. USA.
- 09:50 AM      **COFFEE BREAK**

**FRIDAY, AUGUST 4, 1995**

**Session II: MARINE AQUATIC TOXINS**

**Chairpersons: Dr. Mark Poli and D. Baden**

- 10:10 AM THE DISCOVERY OF DOMOIC ACID AS A NEW MARINE TOXIN.** Jeffrey Wright, National Research Council of Canada.-No Abstract
- 11:00 AM SEAFOOD TOXINS:OUTBREAKS, RESEARCH, AND MANAGEMENT.** Sherwood Hall, Office of Seafood, U.S. Food and Drug Administration HFS-426, Washington, D.C. USA.
- 11:20 AM DEVELOPMENT OF A PROTEIN PHOSPHATASE BASED ASSAY FOR THE DETECTION OF PHOSPHATASE INHIBITORS IN CRUDE WHOLE CELL/ANIMAL EXTRACTS.** Richard E. Honkanen\*<sup>1</sup>, Faith R. Caplan<sup>2</sup>, Gregory M.L. Patterson<sup>2</sup> and Jennifer Abercrombie<sup>1</sup>. <sup>1</sup>Department of Biochemistry and Molecular Biology, University of South Alabama, Mobile, AL and <sup>2</sup>Department of Chemistry, University of Hawaii, Honolulu, HI.
- 11:40 AM ANTIBODIES TO MAITOTOXIN ELICITED BY IMMUNIZATION WITH TOXIN FRAGMENT CONJUGATES.** Gary Bignami - No Abstract
- 12:00 LUNCH AND POSTER REVIEW**

**FRIDAY, AUGUST 4, 1995**

**Session III: MARINE AQUATIC TOXINS**

**Chairperson: Dr. G. Escalona de Motta**

- 1:30 PM**                      **EFFECTS ON FISHERIES AND HUMAN HEALTH LINKED TO A TOXIC ESTUARINE DINOFLAGELLATE.** J.M. Burkholder\* and H.B. Glasgow, Jr. North Carolina State University, Raleigh, NC. USA.
- 2:20 PM**                      **IDENTIFICATION OF A SODIUM CHANNEL TOXIN FROM A CARIBBEAN BENTHIC DINOFLAGELLATE.** G. Escalona de Motta\*, A.L. Rivera-Rentas, J.A. Mercado, T. Tosteson<sup>1</sup> and I. Gonzalez<sup>1</sup>. University of Puerto Rico, Institute of Neurobiology and Department of Biology, San Juan and <sup>1</sup>Department of Marine Sciences, Mayaguez, Puerto Rico.
- 2:40 PM**                      **ALKALOID NEUROTOXINS FROM CARIBBEAN SPONGES OF THE GENUS AGELAS: EFFECT ON NEURONAL MEMBRANE CHANNELS.** A.L. Rivera-Rentas\*, R. Rosa, A.D. Rodriguez<sup>1</sup>, W. Silva<sup>2</sup> and G. Escalona de Motta. University of Puerto Rico Institute of Neurobiology and Departments of Biology and <sup>1</sup>Chemistry, San Juan and Universidad Central del Caribe School of Medicine <sup>2</sup>Department of Pharmacology, Bayamon, Puerto Rico.
- 3:00 PM**                      **THE SEA ANEMONE ANEMONIA SULCATA IS A RICH SOURCE OF BIOLOGICALLY ACTIVE POLYPEPTIDES.** Laszlo Beress. Institute of Toxicology, Christian-Albrechts-University, Kiel, Germany.
- 3:20 PM**                      **COFFEE BREAK**

**FRIDAY, AUGUST 4, 1995**

**Session IV: MARINE AQUATIC TOXINS**

**Chairperson: Dr. M. J. Holmes**

- 3:40 PM Title To Be Announced- Dan Baden - No Abstract
- 4:30 PM **CONFORMATIONAL ANALYSIS OF MARINE NEUROTOXINS AND A HYPOTHESIS CONCERNING THE COMMON PHARMACOPHORES.** Kathleen S. Rein. University of Miami, Rosentstiel School of Marine and Atmospheric Science, Miami, FL. USA.
- 4:50 PM **CARIBBEAN G. TOXICUS: PURIFICATION AND CHARACTERIZATION OF CIGUATERA RELATED SEAFOOD TOXINS.** Peter D.R. Moeller\*, Sharon E. Davis, Stewart M. Knoepp and Bernie A. Lanoue. Marine Biotoxins Program, NOAA Southeast Fisheries Science Center, Charleston, SC. USA.
- 5:10 PM **STRAIN-DEPENDENT PRODUCTION OF TOXINS BY BENTHIC DINOFLAGELLATES.** M.J. Holmes. Bioscience Centre, National University of Singapore, Singapore.
- 5:25 PM **CLOSING REMARKS**



MONDAY, JULY 31 - TUESDAY, AUGUST 1, 1995

POSTER SESSION - SNAKE TOXINS

Chairpersons: Dr. Paul Ried, Dr. M. Kini and Dr. L. Simioni

Poster#/Page#

- 1/131      **THE COMPUTER ANALYSIS OF AMINO ACID SEQUENCES OF TOXIC AND NONTXIC PHOSPHOLIPASES A<sub>2</sub>.** A.N. Vagin<sup>1</sup>, O.N. Vagina<sup>1</sup> and I.I. Parilis<sup>\*2</sup>. <sup>1</sup>Tashkent State University and <sup>2</sup>Institute of Biochemistry, Tashkent, Uzbekistan.
- 2/132      **COMPARISON OF VARANUS GRIZEUS PHOSPHOLIPASE A WITH VENOM ENZYMES.** A. Khafizon, I.I. Parilis\*, E. Kazanov, M. Akramov, R.S. Salikhov and D.Kh. Khamidov. Institute of Biochemistry, Tashkent, Uzbekistan.
- 3/133      **GENOMES OF VIPERIDAE SNAKES CONTAIN ARTIODACTYLA SPECIFIC ART-2 RETROPOSON.** D. Kordis and F. Gubensek\*. Department of Biochemistry and Molecular Biology, Jozef Stefan Institute, Ljubljana, Slovenia.
- 4/134      **EFFECTS OF CROTOXIN ON MURINE OMOHYOID MUSCLE: SPECTROFLUORIMETRY, FLUORESCENCE IMAGING, AND CREATINE KINASE RELEASE.** Paulo A. Melo<sup>\*1,3</sup>, Craig F. Burns<sup>2</sup>, Charlotte L. Ownby<sup>1</sup> and James T. Blankemeyer<sup>2</sup>. <sup>1</sup>Department of Physiological Sciences and <sup>2</sup>Zoology, Oklahoma State University, Stillwater, OK and <sup>3</sup>Departamento de Farmacologia Basica e Clinica, Rio de Janeiro, Brazil.
- 5/135      **THE EFFECT OF ANTIBODIES DIRECTED AGAINST THE CROTOXIN ON THE FROG NERVE-MUSCLE PREPARATION.** I. Horvat-Znidarsic<sup>\*1</sup>, V. Choumet<sup>2</sup>, C. Bon<sup>2</sup> and D. Suput<sup>1</sup>. <sup>1</sup>Institute of Pathophysiology, School of Medicine, Ljubljana and <sup>2</sup>Unite des Venins, Institut Pasteur, Paris, France.
- 6/136      **BETA TAIPOXIN AS A NOVEL MITOGEN HAVING REGENERATIVE WOUND HEALING PROPERTY.** Binie Ver Lipps. Ophidia Products, Houston, TX. USA.

- 7/137 **BIOCHEMICAL AND NEUROTOXICOLOGICAL CHARACTERIZATION OF PARADOXIN, A  $\alpha$ -NEUROTOXIN FROM AUSTRALIAN INLAND TAIPAN SNAKE (OXYURANUS MICROLEPIDOTUS) VENOM.** F. Dorandeu\*, I. Pernot-Marino, G. Lallement. Centre de Recherches du Service de Sante des Armees, Unite de Neurotoxicologie, La Tronche Cedex, France.
- 8/138 **INDUCTION OF GIANT MINIATURE END-PLATE POTENTIALS DURING NEUROMUSCULAR BLOCKADE BY THE PRESYNAPTIC SNAKE NEUROTOXIN TEXTILOTOXIN.** G.M. Nicholson\* and H.I. Wilson. Department of Health Sciences, University of Technology, Sydney, Australia.
- 9/139 **LOCALIZATION AND EXPRESSION OF TRIMERESURUS FLAVOVIRIDIS PHOSPHOLIPASES  $A_2$  IN VENOM GLAND.** Tomohisa Ogawa\*<sup>1</sup>, Hitoshi Onoue<sup>2</sup>, Kazunori Nakagawa<sup>3</sup>, Shintarou Nomura<sup>2</sup>, Katsuo Sueishi<sup>3</sup>, Shosaku Hattori<sup>4</sup>, Hiroshi Kihara<sup>5</sup>, Motonori Onho<sup>1</sup>. <sup>1</sup>Laboratory of Biochemistry, Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka, <sup>2</sup>Department of Pathology, Osaka University Medical School, <sup>3</sup>Laboratory of Pathology I, Faculty of Medicine, Kyushu University, Fukuoka, <sup>4</sup>Institute of Medical Science, University of Tokyo, Kagoshima and <sup>5</sup>Biotech. Research Laboratory, Takara Shuzo Co. Ltd., Shiga, Japan.
- 10/140 **THE NEPHROTOXIC ACTION OF THE CRUDE BOTHROPS INSULARIS VENOM AND OF  $PLA_2$ -CONTAINING A FRACTION.** A.C.F. D Abreu<sup>1</sup>, L. Rodrigues-Simioni<sup>2</sup>, J.C. Cogo<sup>2</sup> and M.A. Cruz-Hofling\*<sup>1</sup>. <sup>1</sup>Department of Histology and Embryology, Institute of Biology, UNICAMP, and <sup>2</sup>Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, Campinas, Brazil.
- 11/141 **NEUROMUSCULAR EFFECTS OF A FRACTION WITH PHOSPHOLIPASE  $A_2$  ACTIVITY ISOLATED FROM BOTHROPS INSULARIS VENOM.** J.C. Cogo\*<sup>1</sup>, J. Prado-Franceschi<sup>2</sup>, A. Pinto-Corrado<sup>3</sup>, J.R. Giglio<sup>4</sup>, J.L. Donato<sup>2</sup> and L. Rodrigues-Simioni<sup>2</sup>. <sup>1</sup>Department of Physiology and Biophysics, I.B., UNICAMP, Campinas, Brazil, <sup>2</sup>Department of Pharmacology, F.C.M., UNICAMP, and Departments of <sup>3</sup>Pharmacology and <sup>4</sup>Biochemistry, FM-USP, Ribeirao Preto, Brazil.

- 12/142 HEPATIC MORPHOLOGICAL ALTERATIONS INDUCED BY BOTHROPS INSULARIS VENOM AND A FRACTION WITH PLA<sup>2</sup>, ACTIVITY IN CHICKS.** C.C. Paronetto<sup>1</sup>, J.C. Cogo<sup>2</sup>, L. Rodrigues-Simioni<sup>2</sup> and M.A. Cruz-Hofling<sup>\*1</sup>. <sup>1</sup>Department of Histology and Embryology, Institute of Biology, UNICAMP, and <sup>2</sup>Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, Campinas, Brazil.
- 13/143 SPECIFICITIES OF Asp49, Asp35 AND Lys49 PHOSPHOLIPASE A<sub>2</sub> ENZYMES ON SKELETAL MUSCLE LIPID SUBSTRATES.** J.E. Fletcher<sup>\*1</sup>, M-S. Jiang<sup>1</sup>, H.S. Selistre de Araujo<sup>2,3</sup> and C.L. Ownby<sup>2</sup>. <sup>1</sup>Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA, <sup>2</sup>Oklahoma State University, Stillwater, OK and <sup>3</sup>Universidade Federal de Sao Carlos, Sao Carlos SP, Brazil.
- 14/144 INHIBITION OF SYNAPTOSOMAL BINDING OF <sup>125</sup>I-DABOITOXIN BY PYTHON SERUM.** M.M. Thwin<sup>\*1</sup>, P. Gopalakrishnakone<sup>1</sup>, R. Yuen<sup>2</sup> and C.H. Tan<sup>2</sup>. Venom and Toxin Research Group, Departments of <sup>1</sup>Anatomy and <sup>2</sup>Biochemistry, Faculty of Medicine, National University of Singapore, Singapore.
- 15/145 THE SCREENING OF BOTHROPS VENOMS FOR NEUROTOXIC ACTIVITY USING THE CHICK BIVENTER CERVICIS PREPARATION.** S.R. Zamuner, J. Prado-Franceschi and L. Rodrigues-Simioni\*. Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, Campinas, Brazil.
- 16/146 NATURE OF THE POSTSYNAPTIC ACTION OF CROTOXIN AT THE GUINEA-PIG END-PLATE.** O. Vital Brazil and M.D. Fontana\*. Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, SP, Brazil.
- 17/147 ELECTROPHYSIOLOGICAL AND ULTRASTRUCTURAL EFFECTS OF MICRURUS NIGROCINCTUS VENOM ON THE NEUROMUSCULAR JUNCTION.** F.C. Goularte<sup>1</sup>, M.A. Cruz-Hofling<sup>2</sup> and Rodrigues-Simioni<sup>\*1</sup>. <sup>1</sup>Department of Pharmacological Sciences, CCBPS, UNIMEP, Piracicaba, SP, Brazil, Departments of <sup>2</sup>Histology and Embryology and <sup>3</sup>Pharmacology, UNICAMP, Campinas, SP, Brazil.

- 18/148      **MODE OF ACTION OF DUVERNOY S GLAND EXTRACTS FROM THE COLUBRIDAE DRYADOPHIS BIFOSSATUS IN THE CHICK BIVENTER CERVICIS NERVE-MUSCLE PREPARATION.** M.D. Fontana\*, M.G. Heleno and O. Vital Brazil. Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, SP, Brazil.
- 19/149      **PHARMACOKINETICS OF <sup>125</sup>I-LABELLED WALTERINNESIA AEGYPTIA VENOM: FLASH DISTRIBUTION OF THE VENOM AND ITS TOXIN.** M. Ismail, M.A. Abd- Elsalam and M.S. Al-Ahaidib\*. The Antivenom and Vaccine Production Center, King Fahad National Guard Hospital, Riyadh, Saudi Arabia.
- 20/150      **ELECTROSPRAY- & MALDI-MS INVESTIGATIONS ON SNAKE TOXINS.** T. Krishnamurthy\*, M. Prabhakaran and S.R. Long. U. S. Army Edgewood RD & E Center, Aberdeen Proving Ground, MD. USA.
- 21/151      **MODULATION OF CHOLINERGIC AND GLUTAMATERGIC NEUROTRANSMISSION IN RAT HIPPOCAMPUS BY  $\alpha$ -DENDROTOXIN AND THE EFFECT OF THE NON-NMDA RECEPTOR ANTAGONIST DNQX.** F. Dorandeu\*<sup>1</sup>, J. Wetherell<sup>2</sup>, I. Pernot-Marino<sup>1</sup>, J.E.H. Tattersall<sup>2</sup>, G. Lallement<sup>1</sup> and P. Fosbraey<sup>2</sup>. <sup>1</sup>Centre de Recherches du Service de Sante des Armees, Unite de Neurotoxicologie, La Tronche Cedex, France and <sup>2</sup>Chemical and Biological Defence Establishment, Salisbury, Wiltshire, UK. USA.
- 22/152      **MORPHOLOGICAL CHARACTERISTICS OF MYOGLOBINURIC RENAL DAMAGE AFTER PSEUDECHIS AUSTRALIS ENVENOMATION.** D. Ponraj and P. Gopalakrishnakone\*. Venom and Toxin Research Group, Department of Anatomy, Faculty of Medicine, National University of Singapore, Singapore.
- 23/153      **ISOLATION AND CHARACTERIZATION OF A PLA<sub>2</sub> MYOTOXIN FROM PRAIRIE RATTLESNAKE (CROTALUS VIRIDIS VIRIDIS) VENOM.** Charlotte L. Ownby\* and Terry R. Colberg. Department of Physiological Sciences, Oklahoma State University, Stillwater, OK. USA.

- 24/154**      **DIFFERENT SENSITIVITY OF WHITE AND RED SKELETAL MUSCLES TO THE MYOTOXIC COMPONENTS OF SNAKE VENOMS.** Paulo A. Melo<sup>\*1,2</sup> and Charlotte L. Ownby<sup>1</sup>. <sup>1</sup> Department of Physiological Sciences, Oklahoma State University, Stillwater, OK and <sup>2</sup>Departamento de Farmacologia Basica e Clinica, Rio de Janeiro, Brazil.
- 25/155**      **EXPRESSION OF RECOMBINANT ACL MYOTOXIN IN E.COLI.** Heloisa S. Selistre De Araujo<sup>\*1,2</sup> and Charlotte L. Ownby<sup>1</sup>. <sup>1</sup>Department of Physiological Sciences, Oklahoma State University, Stillwater, OK and <sup>2</sup>Departamento de Ciencias Fisiologicas, Universidade Federal de Sao Carlos, Sao Carlos, Sao Paulo, Brazil.
- 26/156**      **ALTERED CALCIUM REGULATION IN RESPONSE TO SNAKE MYOTOXINS - CROTAMINE AND CARDIOTOXIN.** M.D. Hubert\*, M-S. Jiang and J.E. Fletcher. Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA. USA.
- 27/157**      **PROPORTIONAL DECREASE OF SARAFOTOXIN SYNTHESIS FOLLOWING EMPTYING OF THE VENOM GLAND OF ATRACTASPIS ENGADDENSIS.** A. Bdolah\*, S. Kinamon and E. Kochva. Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.
- 28/158**      **BRADYKININ POTENTIATING PEPTIDES FROM SNAKE VENOMS AND THEIR SPATIAL STRUCTURES.** Luiza A.F. Ferreira, Departamento de Bioquimica and Biofisica, Instituto Butantan, Sao Paulo, Brasil and Gerhard G. Habermehl\*, Department of Chemistry, School of Veterinary Medicine, Hannover, Germany.
- 29/159**      **THE EFFECTS OF *BOTHROPS ERYTHROMELAS* VENOM (BEV) ON MEAN ARTERIAL BLOOD PRESSURE (MABP) OF ANESTHETIZED DOGS. INVOLVEMENT OF BRADYKININ.** A. Zappellini, A. Lelis and J. Prado-Franceschi\*. Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil.
- 30/160**      **STUDY OF INFLAMMATORY EVENTS INDUCED BY BOTHROPS JARARACA VENOM (BjV) IN THE RAT AIR POUCH.** C.F.P. Teixeira<sup>\*1</sup>, J.W.M. Costa-Cruz<sup>1</sup>, Y. Cury<sup>2</sup>. <sup>1</sup>Laboratorio de Farmacologia and <sup>2</sup>Laboratorio de Fisiopatologia, Instituto Butantan, Sao Paulo, Brazil.

- 31/161      **STUDIES ON ANTICOMPLEMENTARY, PROTEOLYTIC AND HEMOLYTIC ACTIVITY OF VIPERA AMMODYTES VENOM.** S.A. Stanilova<sup>1</sup>, I.B. Matev<sup>2</sup> and Z.D. Zhelev\*<sup>1</sup>. <sup>1</sup>Department of Molecular Biology and Immunology, Medical University, and <sup>2</sup>Laboratory of Herpetology, Higher Institute of Veterinary Medicine and Zootechnics, Bulgaria.
- 32/162      **PRODUCTION OF A MONOCLONAL ANTIBODY AGAINST CRUDE VENOM FROM THE BROAD-BANDED COPPERHEAD (AGKISTRODON CONTORTRIX LATICINCTUS).** Lynda A. Ramos\* and John C. Perez. Biology Department, Texas A&M University- Kingsville, Kingsville, TX. USA.
- 33/163      **IMMUNOLOGICAL RELATEDNESS OF VARIOUS SNAKE VENOMS WITH A MONOCLONAL ANTIBODY.** Elda E. Sanchez\* and John C. Perez. Biology Department, Texas A&M University-Kingsville, Kingsville, TX.
- 34/164      **PARTIAL CHARACTERIZATION AND THE ROLE OF GLYCOSYL MOIETIES OF THE HEMORRHAGIC TOXIN, PROTEINASE H, FROM CROTALUS ADAMANTEUS VENOM.** Steffan G. Anderson\* and Charlotte L. Ownby. Department of Physiological Sciences, Oklahoma State University, Stillwater, OK. USA.
- 35/165      **SCREENING A cDNA LIBRARY FROM CROTALUS ATROX WITH MONOCLONAL ANTIBODIES SPECIFIC FOR HEMORRHAGIC TOXINS IN VENOM.** Sonia K. Lerma\*, Albar Chavana and James R. Pierce. Texas A&M University-Kingsville, Kingsville, TX. USA.
- 36/166      **THE STRUCTURE AND THE FUNCTION OF MONGOOSE ANTIHEMORRHAGIC FACTOR.** K. Yonaha\*<sup>1</sup> and Y. Tomihara<sup>2</sup>. <sup>1</sup>Department of Bioscience and Biotechnology, University of the Ryukyus, Nishihara, Okinawa, Japan and <sup>2</sup>Department of Habu, Okinawa Prefectural Institute of Public Health, Ozato, Okinawa, Japan.
- 37/167      **A SNAKE VENOM DISINTEGRIN PREVENTS REOCCLUSION FOLLOWING THROMBOLYSIS IN A CANINE CAROTID ARTERY THROMBOSIS MODEL AND BLOCKS MELANOMA METASTASIS.** F.S. Markland. Department of Biochemistry and Molecular Biology, University of Southern California, School of Medicine, Los Angeles, CA. USA.

- 38/168 PURIFICATION AND CHARACTERIZATION OF ANTICOAGULANTS FROM AUSTRELAPS SUPERBUS (COPPERHEAD) SNAKE VENOM.** R. Manjunatha Kini. Bioscience Centre, Faculty of Science, National University of Singapore, Singapore.
- 39/169 PURIFICATION AND MOLECULAR CLONING OF THROMBIN-LIKE ENZYME FROM THE VENOM OF AGKISTRODON CALIGINOSUS (KOREAN BULLDOGSA).** Yeong-Shik Kim<sup>\*1</sup>, Kyoung-Youl Yang<sup>1,2</sup>, Eun-Mi Choi<sup>2</sup>, Bum-Soo Hahn<sup>1</sup> and Il-Moo Chang<sup>1</sup>. <sup>1</sup>Natural Products Research Institute, Seoul National University, Seoul and <sup>2</sup>Department of Chemistry, University of Incheon, Incheon, Korea.
- 40/170 FIBROLASE, AN EFFECTIVE THROMBOLYTIC AGENT IN ARTERIAL AND VENOUS THROMBOSIS ANIMAL MODELS.** F.S. Markland. Department of Biochemistry and Molecular Biology, University of Southern California, School of Medicine, Los Angeles, CA. USA.
- 41/171 FIBRINOGEN-CLOTTING ACTIVITY OF PROTEINASE H, THE HEMORRHAGIC ZINC-METALLOPROTEINASE FROM CROTALUS ADAMANTEUS (EASTERN DIAMONDBACK RATTLESNAKE) VENOM.** Steffan G. Anderson\* and Charlotte L. Ownby. Department of Physiological Sciences, Oklahoma State University, Stillwater, OK. USA.
- 42/172 FIBRIN MONOMER GENERATION AND THEIR POLYMERIZATION BY VIPERA RUSSELLI VENOM.** Abid Azhar\*, Fatima Sughra Ausat and Fizza Ahmad. Coagulation and Haemostasis Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan.
- 43/173 EFFECT OF BOTHROPS JARACA (Bj) AND CROTALUS DURISSUS TERRIFICUS (Cdt) VENOM ON NEUTROPHIL MIGRATION. IN VITRO CHEMOTAXIS STUDIES.** S.H.P. Farsky\*, L.R.C. Goncalves, M.C.C. Sousa e Silva and Y. Cury. Laboratorio de Fisiopatologia, Instituto Butantan, Sao Paulo, Brazil.
- 44/174 PARTICIPATION OF ENDOGENOUS CORTICOSTEROIDS IN ACUTE INFLAMMATION (EDEMA) INDUCED BY BOTHROPS JARARACA VENOM (BjV).** Y. Cury<sup>\*1</sup>, S.H.P. Farsky<sup>1</sup> and C.F.P. Teixeira<sup>2</sup>. <sup>1</sup>Laboratorio de Fisiopatologia and <sup>2</sup>Laboratorio de Farmacologia, Instituto Butantan, Sao Paulo, Brazil.

- 45/175 **ENVENOMATION BY SNAKES OF THE GENUS BOTHROPS IN RECIFE, BRAZIL.** M.C. Guarnieri\*<sup>1</sup>, L.R. Andrade<sup>1</sup>, P.K. Lima<sup>1</sup>, D.C.C. Pimentel<sup>1</sup>, K.C. Maia<sup>1</sup>, C.M.L. Vasconcelos<sup>1</sup>, R.C. Valenca<sup>1</sup>, M.L. Porto<sup>2</sup> and A.E. Oliveira Jr.<sup>2</sup>. <sup>1</sup>Departamento de Biofisica e Radiobiologia, Universidade Federal de Pernambuco and <sup>2</sup>CEATOX, Hospital da Restauracao, Recife, Brazil.
- 46/176 **POISONOUS ANIMALS ACCIDENTS IN PERNAMBUCO, BRAZIL: A EPIDEMIOLOGICAL RETROSPECTIVE STUDY FROM 1992 TO 1994.** M.C. Guarnieri\*<sup>1</sup>, D.C.C. Pimentel<sup>1</sup>, K.C. Maia<sup>1</sup>, L.R. Andrade<sup>1</sup>, P.K. Lima<sup>1</sup>, C.M.L. Vasconcelos<sup>1</sup>, R.C. Valeca<sup>1</sup>, M.L. Porto<sup>2</sup> and A.E. Oliveira Jr.<sup>2</sup>. <sup>1</sup>Departamento de Biofisica e Radiobiologia, Universidade Federal de Pernambuco and <sup>2</sup>CEATOX, Hospital da Restauracao, Recife, Brazil.
- 47/177 **NEUTRALIZING CAPACITY OF SPECIFIC AND COMMERCIAL ANTISERA AGAINST CROTALUS DURISSUS TERRIFICUS AND BOTHROPS JARARACUSSU VENOMS AND THEIR MAJOR TOXINS (CROTOXIN AND BOTHROSPTOXIN).** Y.O. Franco, J. Prado-Franceschi\* and L. Rodrigues-Simioni. Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, Campinas, Brazil.
- 48/178 **CLINICAL ASPECTS AND TREATMENT OF ACCIDENTS BY GENUS CROTALUS.** M.C. Guarnieri\*<sup>1</sup>, K.C. Maia<sup>1</sup>, L.R. Andrade<sup>1</sup>, P.K. Lima<sup>1</sup>, D.C.C. Pimentel<sup>1</sup>, R.C. Valenca<sup>1</sup>, C.M.L. Vasconcelos<sup>1</sup>, M.L. Porto<sup>2</sup> and A.E. Oliveira Jr.<sup>2</sup>. <sup>1</sup>Departamento de Biofisica e Radiobiologia, Universidade Federal de Pernambuco and <sup>2</sup>CEATOX, Hospital da Restauracao, Recife, Brazil.
- 49/179 **NEW APPROACHES IN ANTIVENOM THERAPY.** F. Audebert<sup>1</sup>, C. Bon\*<sup>1</sup>, V. Choumet<sup>1</sup>, G. Riviere<sup>1</sup>, A. Robbe-Vincent<sup>1</sup>, M. Sorkine<sup>1</sup>, A. Sabouraud<sup>2</sup>, J.-M. Scherrmann<sup>2</sup> and M. Urtizberea<sup>2</sup>. <sup>1</sup>Unite des Venins, Institut Pasteur, Paris, France and <sup>2</sup>Unite INSERM U<sup>2</sup>6, Hopital Fernand Widal, Paris, France.
- 50/180 **MTx1 AND 2 FROM GREEN MAMBA VENOM ALLOSTERICALLY ACTIVATE M1 AND BLOCK M<sup>3</sup> MUSCARINIC RECEPTORS.** D. Jerusalinsky<sup>1</sup>, E. Kornisiuk<sup>1</sup>, S. Habtemariam<sup>2</sup> and A. Harvey<sup>2</sup>. <sup>1</sup>IBCN, Faculty of Medicine, University Buenos Aires, Argentina and <sup>2</sup>Department of Physiology and Pharmacology, SIDR, University of Strathclyde, Glasgow, UK.



**MONDAY, 31 JULY- TUESDAY 1 AUGUST, 1995**

**POSTER SESSION - BACTERIAL TOXINS**

**Chairperson: Dr. John Hewetson**

**Poster#/Page#**

- 51/181      A SENSITIVE AND SPECIFIC IN-SITU MODEL TO STUDY THE PHARMACOLOGICAL MECHANISMS OF AGENTS/TOXINS.** J. von Bredow, J. Vick\*, H. Lownsohn and N. Adams. Walter Reed Army Institute of Research, Washington, D.C. USA.
- 52/182      KINETIC ISOTOPE EFFECT STUDIES ON THE TRANSITION STATE OF PERTUSSIS TOXIN.** J. Scheuring\* and V.L. Schramm. Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY. USA.
- 53/183      DETERMINATION OF THE MINIMUM CYTOTOXIC CLOSTRIDIUM PERFRINGENS ENTEROTOXIN FRAGMENT THROUGH DELETION ANALYSIS.** John F. Kokai- Kun\* and Bruce A. McClane. University of Pittsburgh School of Medicine, Pittsburgh, PA. USA.
- 54/184      BIOLOGICAL EFFECTS AND VACCINE POTENTIAL OF STAPHYLOCOCCAL ENTEROTOXIN B MUTANTS: N23K AND F44S.** M.A. Woody\*<sup>1</sup>, T. Krakauer<sup>1</sup>, J. Bill<sup>2</sup> and B.G. Stiles<sup>1</sup>. <sup>1</sup>Toxinology Division, USAMRIID, Frederick, MD and <sup>2</sup>Supragen, Inc., Lakewood, CO. USA.

WEDNESDAY, AUGUST 2 - THURSDAY, AUGUST 3, 1995

POSTER SESSION - PLANT TOXINS AND MYCOTOXINS

Chairperson: Dr. M. Ahmad

Poster#/Page#

- 55/185**      **CARRY OVER OF AFLATOXINS IN MILK AND MILK PRODUCTS IN PAKISTAN AND THEIR POSSIBLE CONTROL.** Mansoor A. Ahmad\*, Butool A. Khan, Zuzzer A. Shamsuddin and Mobeen A. Khan. Mycotoxins Laboratory, PCSIR Laboratories Complex, Karachi, Pakistan.
- 56/186**      **FUNGAL CONTAMINATION OF MEDICINAL PLANT PRODUCTS AND AFLATOXINS DETECTION.** I. Rizzo\*, G. Vedoya, E. Varsavsky and H. Frade. Instituto Nacional de Medicamentos, Capital Federal, Argentina.
- 57/187**      **METABOLIC BASIS OF THE PROTECTIVE EFFECT OF SOME FOOD INGREDIENTS AGAINST DEOXYNIVALENOL TOXICITY.** L.V. Kravchenko\*, O.L. Oboljsky, L.P. Zacharova and V.A. Tutelyan. Institute of Nutrition, Moscow, Russia.
- 58/188**      **MACROCYCLIC TRICHOTECENES IN ARGENTINEAN BACCHARIS CORIDIFOLIA AND ARTHEMISIOIDES.** I. Rizzo\*, E. Varsavsky and H. Frade. Instituto Nacional de Medicamentos, Capital Federal, Argentina.
- 59/189**      **TOXICOKINETICS OF TRITIATED DIHYDROMICROCYSTIN-LR IN SWINE.** R.R. Stotts\*<sup>1</sup>, R.A. Twardock<sup>2</sup>, G.D. Koritz<sup>2</sup>, W.M. Haschek<sup>2</sup>, R.K. Manuel<sup>2</sup>, W.L. Hollis<sup>2</sup> and V.R. Beasley<sup>2</sup>. <sup>1</sup>U.S. Army Medical Institute of Chemical Defense, Aberdeen Proving Ground, MD and <sup>2</sup>University of Illinois College of Veterinary Medicine, Urbana, IL. USA.
- 60/190**      **DESIGN OF INHIBITORS FOR RICIN A-CHAIN.** Xiangyang Chen\*, Todd Link and Vern L. Schramm. Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY. USA.
- 61/191**      **FUMONISIN B1 ALTERS THYMUS FUNCTION IN MICE.** Elena A. Martinova. Institute of Nutrition, Moscow, Russia.

62/192

**SENSITIVITY OF  $\alpha$ - AMANITIN TO OXIDATION BY A SYSTEM OF LACTOPEROXIDASE AND HYDROGEN PEROXIDE.** A.M. Zheleva<sup>1</sup>, Z.D. Zhelev\*<sup>2</sup>, D. Michelot<sup>3</sup> and N.E. Lazarov<sup>4</sup>. <sup>1</sup>Department of Chemistry and Biochemistry, <sup>2</sup>Department of Molecular Biology and Immunology, <sup>4</sup>Department of Anatomy, Histology and Embryology, Medical University, Bulgaria and <sup>3</sup>Laboratoire de Chimie, Museum National d'Histoire Naturelle, Paris, France.

WEDNESDAY, AUGUST 2 - THURSDAY, AUGUST 3, 1995

Poster Session: SCORPIONS AND OTHER ARTHROPODS

Chairpersons: Dr. C. Garcia and Dr. D. Creasia

Poster #/Page#

- 63/193**      **INCORPORATION OF A 35 kDa PURIFIED PROTEIN FROM LOXOSCELES VENOM TRANSFORMS HUMAN ERYTHROCYTES INTO ACTIVATORS OF AUTOLOGOUS COMPLEMENT ALTERNATIVE PATHWAY.** D.V. Tambourgi, F.C. Magnoli, V.R.D. Von Eickstedt, Z.C. Benedetti<sup>1</sup>, V.L. Petricevich and W. Dias da Silva\*. Instituto Butantan and <sup>1</sup>University de Sao Paulo, Sao Paulo, Brazil.
- 64/194**      **A COMPARATIVE BIOCHEMICAL STUDY OF COMPONENTS WITH LETHAL AND DERMONECROTIC ACTIVITIES FROM THE VENOM OF SPIDERS OF THE GENUS LOXOSCELES.** K.C. Barbaro\*<sup>1</sup>, L. Morhy<sup>2</sup>, M.V. Sousa<sup>2</sup>, V.R.D. Eickstedt<sup>1</sup> and I. Mota<sup>1</sup>. <sup>1</sup>Instituto Butantan, Sao Paulo and <sup>2</sup>Centro Brasileiro de Sequenciamento de Proteinas, Universidade de Brasilia, Brazil.
- 65/195**      **REGULATION OF STORAGE PROTEIN PRODUCTION IN ENVENOMATED HOST LARVAE PARASITIZED BY EUPLECTRUS SPP. (HYMENOPTERA: EULOPHIDAE).** Sandra L. Brandt\*<sup>1</sup>, Thomas A. Coudron<sup>1</sup>, Doug Jones<sup>2</sup> and Abdur Raquib<sup>3</sup>. <sup>1</sup>USDA- ARS-Biological Control of Insects Research Laboratory, Columbia, MO, <sup>2</sup>Graduate Center for Toxicology, University of Kentucky, Lexington, KY and <sup>3</sup>Department of Entomology, University of Missouri, Columbia, MO. USA.
- 66/196**      **CLONING AND EXPRESSION OF TOXIN Cn 5 FROM THE SCORPION CENTRUROIDES NOXIUS HOFFMANN.** C. Garcia\*<sup>1</sup>, E.S. Calderon-Aranda<sup>2</sup>, F. Bolivar<sup>1</sup>, L.D. Possani<sup>1</sup> and B. Becerril<sup>1</sup>. <sup>1</sup>Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Avenida Universidad, Cuernavaca, Mexico and <sup>2</sup>Centro de Biotecnologia, Universidad Autonoma Estado Morelos, Cuernavaca, Mexico.

- 67/197**      **AMINO ACID SEQUENCE OF *TITYUS DISCREPANS* SCORPIONS TOXINS.** G.D. D'Suze\*<sup>1</sup>, F. Corona<sup>2</sup>, C. Sevcik<sup>1</sup> and L. Possani<sup>2</sup>. <sup>1</sup>Instituto Venezolano de Investigaciones Cientificas, Centro de Biofisica y Bioquimica, Laboratory of Cellular Neuropharmacology, Caracas, Venezuela and <sup>2</sup>Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico.
- 68/198**      **ANTIGENIC CHARACTERIZATION OF TOXIN 2 FROM DE VENOM OF THE SCORPION *CENTRUROIDES NOXIUS* HOFFMANN.** G. Gurrola-Briones\*, A. Mendez- Teapila and L.D. Possani. Department of Molecular Recognition and Structural Biology, Instituto de Biotecnologia-Universidad Nacional Autonoma de Mexico, Avenida Universidad, Cuernavaca, Mexico.
- 69/199**      **ISOLATION AND CHARACTERIZATION OF INSECT-SPECIFIC PEPTIDE TOXINS FROM THE VENOM OF THE SOUTH INDIAN RED SCORPION, *BUTHUS TAMULUS*.** Wudayagiri Rajendra\*<sup>1</sup>, Haim Moscovitz<sup>2</sup>, Rafael Hermann<sup>2</sup>, Prabhakar V. Choudary<sup>2</sup> and Bruce D. Hammock<sup>2</sup>. <sup>1</sup>Department of Zoology, Sri Venkateswara University, A.P., India and <sup>2</sup>Department of Entomology and Environmental Toxicology, University of California, Davis, CA. USA.
- 70/200**      **ISOLATION OF A PURIFIED TOXIN (MARTOXIN, MTX) FROM THE VENOM OF THE SCORPION *BUTHUS MARTENSI* KARSCH WITH ADRENERGIC AND NANC ACTIONS.** Gong Jianping<sup>1</sup>, R. Manjunatha Kini<sup>2</sup>, M.C.E. Gwee<sup>3</sup> and P. Gopalakrishnakone\*<sup>1</sup>. Venom and Toxin Research Group, Departments of <sup>1</sup>Anatomy and <sup>3</sup>Pharmacology, Faculty of Medicine, and <sup>2</sup>Bioscience Centre, Faculty of Science, National University of Singapore, Singapore.
- 71/201**      **PURIFICATION, CHARACTERIZATION AND GENOMIC ORGANIZATION OF KTX2 FROM *ANDROCTONUS AUSTRALIS*, A NEW INHIBITOR OF VOLTAGE AND CALCIUM ACTIVATED K<sup>+</sup> CHANNEL.** F. Laraba-Djebari, C. Legros, M. Crest<sup>1</sup>, B. Ceard, R. Romi, P. Mansuelle, G. Jacquet<sup>1</sup>, J. Van Rietchoten, M. Gola<sup>1</sup>, H. Rochat, P.E. Bougis P.E. and M.F. Martin-Eauclaire\*. Laboratoire de Biochimie, Ingenierie des Proteines, Unite de Recherche Associee, Marseille, France and <sup>1</sup>Laboratoire de Neurobiologie, Centre National de la Recherche Scientifique, Marseille, France.

- 72/202       **$\alpha$ -LATROTOXIN CHANGES THE PHOSPHORYLATION OF SYNAPTOSOMAL PROTEIN P65.** N.H. Himmelreich\*, L.G. Storchak and N.G. Pozdnyakova. A. V. Palladin Institute of Biochemistry, Unkranian Academy of Sciences, Kiev, Ukraine.
- 73/203      **POTENTIAL-DEPENDENCE OF INTERACTION OF  $\alpha$ -LATROTOXIN CHANNELS IN PLANAR LIPID MEMBRANE WITH ANTIBODIES TO  $\alpha$ -LATROTOXIN AND THE EFFECTS OF PRONASE.** A.N. Chanturiya<sup>\*1</sup>, O.Ya. Shatursky<sup>2</sup>, A.N. Nikolaenko<sup>2</sup> and V.K. Lishko<sup>2</sup>. <sup>1</sup>LTPB/NICHD - National Institutes of Health, Bethesda, MD and <sup>2</sup>A.V. Palladin Institute of Biochemistry, Kiev, Ukraine.
- 74/204      **CLINICAL ASPECTS AND TREATMENT OF SCORPIONS STINGS IN THE REGION OF RECIFE, BRAZIL.** M.C. Guarnieri<sup>\*1</sup>, P.K. Lima<sup>1</sup>, D.C.C. Pimentel<sup>1</sup>, K.C. Maia<sup>1</sup>, L.R. Andrade<sup>1</sup>, R.C. Valenc<sup>1</sup>, C.M.L. Vasconcelos<sup>1</sup>, M.L. Porto<sup>2</sup> and A.E. Oliveira Jr.<sup>2</sup>. <sup>1</sup>Departamento de Biofisica e Radiobiologia, Universidade Federal de Pernambuco and <sup>2</sup>CEATOX, Hospital da Resauracao, Recife, Brazil.
- 75/205      **NEUROTRANSMITTER SECRETION STIMULATED BY  $\alpha$ -LTX ACTIVATES PHOSPHORYLATION OF PKC SUBSTRATES IN PRIMARY NEURONES.** Delio Mercanti<sup>\*1</sup>, Maria Teresa Ciotti<sup>1</sup>, Marta Hribal<sup>1</sup>, Luisa Milazzo<sup>1</sup>, Patrizia Casalbore<sup>2</sup> and Alfonso Grasso<sup>2</sup>. <sup>1</sup>Institute of Neurobiology and <sup>2</sup>Institute of Cell Biology, Rome, Italy.
- 76/206       **$\alpha$ -LATROTOXIN-INDUCED FUSION OF NEGATIVELY CHARGED LIPOSOMES.** Ya. T. Terletskaia\* and I.O. Triakash. Palladin Institute of Biochemistry, Ukrainian Academy of Sciences, Kiev, Ukraine.
- 77/207      **MICROASSAYS FOR THE INVESTIGATIONS OF BIOLOGY ACTIVE COMPOUNDS IN SMALL-SCALE VENOM SAMPLES.** P. Escoubas\*, T. Nakajima. Suntory Institute for Bioorganic Research, Osaka, Japan.

WEDNESDAY, AUGUST 2 - THURSDAY, AUGUST 3, 1995

POSTER SESSION - MARINE VENOMS

Chairperson: Dr. J.C. Freitas

Poster#/Page#

- 78/208      **A CASE OF ENVENOMING BY PORTUGUESE MAN-O'WAR FROM THE BRAZILIAN COAST.** J.C. Freitas<sup>\*1</sup>, W.A. Schiozer<sup>2</sup> and E.L.A. Malpezzi<sup>1</sup>. <sup>1</sup>Biosciences Institute and Marine Biology Center, University of Sao Paulo, Sao Paulo, Brazil and <sup>2</sup>University Hospital of University of Sao Paulo, Sao Paulo, Brazil.
- 79/209      **THE GILLS OF FISH AND CRUSTACEANS: VULNERABILITY AND RESISTANCE TO CYTOLYTIC TOXINS.** D. Mebs<sup>\*1</sup>, C. Giese<sup>2</sup> and B. Werding<sup>2</sup>. <sup>1</sup>Zentrum der Rechtsmedizin, University of Frankfurt, Frankfurt, Germany and <sup>2</sup>Institute fur Allgemeine und Spezielle Zoologie, University of Giessen, Federal Republic of Germany.
- 80/210      **TRIMETHYL SULFONIUM CHLORIDE ISOLATED FROM MIDGUT GLAND OF APLYSIA BRASILIANA PREVENTS THE VAGUS NERVE EFFECTS ON THE TOAD'S HEART.** J.C. Freitas<sup>\*1</sup>, A. Freitas<sup>2</sup> and N.F. Roque<sup>2</sup>. <sup>1</sup>Biosciences Institute and Marine Biology Center, University of Sao Paulo, Sao Paulo, Brazil and <sup>2</sup>Chemistry Institute, University of Sao Paulo, Sao Paulo, Brazil.
- 81/211      **EXPRESSION OF THE GENE ENCODING THE -SUBUNIT OF STONUSTOXIN FROM STONEFISH, SYNANCEJA HORRIDA.** F.G. Ghadessy, K. Jeyaseelan, H.E. Khoo and R. Yuen<sup>\*</sup>. Department of Biochemistry, Faculty of Medicine and the Bioscience Centre, National University of Singapore, Kent Ridge Crescent, Singapore.
- 82/212      **MORPHOLOGICAL CHANGES CAUSED BY STONUSTOXIN IN SKELETAL AND SMOOTH MUSCLE.** K.S.Y. Low<sup>\*1</sup>, P. Gopalakrishnakone<sup>2</sup>, M.C.E. Gwee<sup>1</sup>, R. Yuen<sup>3</sup> and H.E. Khoo<sup>3</sup>. Venom and Toxin Research Group, Departments of <sup>1</sup>Pharmacology, <sup>2</sup>Anatomy and <sup>3</sup>Biochemistry, Faculty of Medicine, National University of Singapore, Singapore.

WEDNESDAY, AUGUST 2 - FRIDAY, AUGUST 4, 1995

POSTER SESSION - MARINE AND AQUATIC TOXINS

Chairpersons: Dr. O. Castaneda and Dr. R. Frangez

Poster#/Page#

- 83/213      **INHIBITION OF MEMBRANE-BOUND ACETYLCHOLINESTERASE BY A NEW AChE INHIBITOR FROM THE ZOANTHID PARAZOANTHUS AXINELLAE.** J. Strupi-Suput<sup>\*1</sup>, L. Kamaric<sup>1</sup>, T. Turk<sup>2</sup> and D. Suput<sup>1</sup>. <sup>1</sup>School of Medicine, Institute of Pathophysiology, EDT Lab, Ljubljana and <sup>2</sup>Department of Biology, BFT, Ljubljana.
- 84/214      **ShK IS A POTASSIUM CHANNEL BLOCKER FROM THE CARIBBEAN SEA ANEMONE STICHODACTYLA HELIANTHUS.** O. Castaneda<sup>\*1</sup>, E.G. Rowan<sup>2</sup>, L. Young<sup>2</sup> and E. Karlsson<sup>3</sup>. <sup>1</sup>Facultad de Biología, Universidad de La Habana, Cuba, <sup>2</sup>Strathelyde Institute for Drug Research, Glasgow, U.K. and <sup>3</sup>Department of Biochemistry, Biomedical Center, Uppsala, Sweden.
- 85/215      **CARDIOVASCULAR EFFECTS OF EQUINATOXIN III.** R. Frangez<sup>\*</sup> and D. Suput. Laboratory for Cell Physiology and Toxinology, Institute of Pathophysiology, University of Ljubljana, School of Medicine, Ljubljana, Slovenia.
- 86/216      **CYTOTOXIC AND NEUROTOXIC EFFECTS INDUCED BY HALITOXIN ISOLATED FROM AMPHIMEDON VIRIDIS (PORIFERA).** J.C. Freitas<sup>\*1,2</sup>, E.L.A. Malpezzi<sup>1,2</sup>, L.V. Costa<sup>1,2</sup>, R.G.S. Berlinck<sup>3,2</sup>, A.M.P. Almeida<sup>3</sup>, C.A. Ogawa<sup>3</sup>, M.A.A. Sanchez<sup>3</sup> and E.M. Hajdu<sup>4</sup>. <sup>1</sup>Biosciences Institute, University of Sao Paulo, Sao Paulo, Brazil, <sup>2</sup>Marine Biology Center, University of Sao Paulo, Sao Sebastiao, Brazil, <sup>3</sup>Chemistry Institute of Sao Carlos, University of Sao Paulo, Sao Paulo, Brazil and <sup>4</sup>Institut voor Systematiek en Populatiebiologie, University of Amsterdam, Amsterdam, Netherlands.



- 87/217 4-AMINOPYRIDINE ANTAGONIZES THE LETHAL EFFECTS OF SAXITOXIN (STX) AND TETRODOTOXIN (TTX).** B.J. Benton, D.L. Spriggs, B.R. Capacio and F.-C.T. Chang. Pathophysiology Division, USAMRICD, APG-EA, MD. USA.
- 88/218 ISOLATION AND IDENTIFICATION OF A KEY METABOLITE FROM 3,4-DIAMINOPYRIDINE ADMINISTRATION TO GUINEA PIGS.** Robert L. von Tersch\*, Benedict R. Capacio, Christopher E. Byers and Fat-Chun T. Chang. Pharmacology and Pathophysiology Divisions, United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD. USA.
- 89/219 11-OXO TETRODOTOXIN AND A SPECIFICALLY LABELLED <sup>3</sup>H-TTX.** B.Q. Wu<sup>1</sup>, C.Y. Kao\*<sup>1</sup>, M. Yotsu<sup>2</sup>, T. Yasumoto<sup>2</sup> and S.R. Levinson<sup>3</sup>. <sup>1</sup>SUNY Downstate Medical Center, Brooklyn, NY, <sup>2</sup>Tohoku University, Sendai, Japan and <sup>3</sup>University of Colorado Medical Center, Denver, CO. USA.
- 90/220 RECEPTOR BINDING ASSAY AND HPLC ANALYSIS OF BREVETOXINS IN ORGANISMS EXPOSED TO A FLORIDA RED TIDE.** T.A. Leighfield\*<sup>1</sup>, M.S. Henry<sup>2</sup>, R.H. Pierce<sup>2</sup>, F.M. Van Dolah<sup>1</sup> and J. Zhou<sup>2</sup>. <sup>1</sup>Marine Biotoxins Program, U.S. National Marine Fisheries Service, Charleston, SC and <sup>2</sup>Mote Marine Laboratory, Sarasota, FL. USA.
- 91/221 COMPARATIVE RESPONSE OF HUMAN AND MURINE CELL LINES BY CELL BIOASSAY TO SODIUM CHANNEL ACTIVE MARINE TOXINS AND EXTRACTS.** Ronald L. Manger\*, Linda S. Leja, Sue Y. Lee, James M. Hungerford and Marleen M. Wekell. U.S. FDA, Seafood Products Research Center, Bothell, WA. USA.
- 92/222 ASSAY OF SAXITOXIN IN SAMPLES FROM HUMAN VICTIMS OF PARALYTIC SHELLFISH POISONING BY BINDING COMPETITION TO SAXIPHILIN AND BLOCK OF SINGLE SODIUM CHANNELS.** P.M Bell\*<sup>1</sup>, B.D. Gessner<sup>2</sup>, S. Hall<sup>3</sup> and E. Moczydlowski<sup>1</sup>. <sup>1</sup>Department of Pharmacology, Yale University School of Medicine, New Haven, CT, <sup>2</sup>Sections of Epidemiology and Maternal, Child and Family Health, Department of Health and Social Services, Anchorage, AK and <sup>3</sup>U.S. Food and Drug Administration, Washington, D.C. USA.

- 93/223      **ANALYSIS OF SAMPLES FROM A HUMAN PSP INTOXICATION EVENT USING A SAXITOXIN RECEPTOR ASSAY AND HPLC.** G.J. Doucette\*<sup>1</sup>, M.M. Logan<sup>1</sup>, F.M. Van Dolah<sup>1</sup> and <sup>2</sup> S. Hall. <sup>1</sup>Marine Biotoxins Program, National Marine Fisheries Service, Charleston, SC and <sup>2</sup>U.S. Food and Drug Administration, Washington, D.C. USA.
- 94/224      **AN OUTBREAK OF PARALYTIC SHELLFISH POISONING IN KODIAK ALASKA:COMPARISON OF TOXIN DETECTION METHODS.** S. Hall<sup>1\*</sup>, B. Gessner<sup>2</sup>, M. Poli<sup>3</sup>, P. Eilers<sup>1</sup>, S. Conrad<sup>1</sup>, E. Waldron<sup>1</sup> and V. Brewer<sup>1</sup>. <sup>1</sup> Office of Seafood, U.S. Food and Drug Administration HFS-426, Washington, D.C. and <sup>2</sup>State of Alaska, <sup>3</sup>Toxinology Division, USAMRIID, Fort Detrick, MD, USA.
- 95/225      **INVESTIGATION OF ALEXANDRIUM SP, ISOLATED FROM KODIAK, ALASKA FOLLOWING AN OUTBREAK OF PARALYTIC SHELLFISH POISONING.** E. Waldron\*<sup>1</sup>, B. Himsbloom<sup>2</sup>, S. Hall<sup>1</sup>, P. Eilers<sup>1</sup>, and S. Conrad<sup>1</sup>, <sup>1</sup> Office of Seafood, U.S. Food and Drug Administration HFS-426, Washington, D.C.<sup>2</sup>Fisheries Industrial Technology Center, University of Alaska, Fairbanks, Alaska, USA.
- 96/226      **FIELD PHYTOPLANKTON OBSERVATIONS AND MARINE BIOTOXIN MANAGEMENT.** S. Conrad\*<sup>1</sup>, G. Langlois<sup>2</sup>, P. Eilers<sup>1</sup>, and S. Hall<sup>1</sup>, <sup>1</sup> Office of Seafood, U.S. Food and Drug Administration HFS-426, Washington, D.C.<sup>2</sup>California Department of Health Services, Berkeley, CA, USA..
- 97/227      **DOMOIC ACID ANALYSIS.** P. Eilers\*, S. Conrad, and S. Hall, Office of Seafood, U.S. Food and Drug Administration HFS-426, Washington, D.C. USA.

## DOUBLE ROLE OF THE CHAPERON SUBUNIT OF CROTOXIN

C. Bon, V. Choumet, E. Délot, G. Faure, A. Robbe-Vincent & B. Saliou  
Unité des Venins, Institut Pasteur, 25 rue du Dr. Roux,  
75724 PARIS, FRANCE.

Crotoxin, the main toxin from the venom of rattlesnake, is a phospholipase A<sub>2</sub> (PLA<sub>2</sub>)  $\beta$ -neurotoxin which blocks neuromuscular transmission. It consists of the non-covalent association of two subunits: a basic and weakly toxic PLA<sub>2</sub> subunit (CB) and a non-toxic, non-enzymatic subunit (CA) which enhances the toxicity of CB. Crotoxin complex (CACB) dissociates upon interaction with membranes: CB binds while CA does not.

Multiple variants of each subunit give different crotoxin complexes characterized by different lethal potencies, different capacities to block neuromuscular transmission and different enzymatic activities ( $K_m$  and  $V_{max}$ ). These differences correlate with the stability of the complexes, the less toxic isoforms are less stable ( $K_d \# 25$  nM) and dissociate rapidly (half-life # 1 min) whereas the more toxic isoforms are more stable ( $K_d \# 4$  nM) and dissociate more slowly (half-life # 15 min). Further, the rate of interaction of crotoxin complexes with vesicles of negatively charged phospholipids parallels the rate of dissociation of the complexes in the absence of vesicles indicating the stability of the complex, by reducing the non specific adsorption of the PLA<sub>2</sub> subunit, plays a major role for the specificity of the action of crotoxin.

Binding studies performed with <sup>125</sup>I-labelled crotoxin indicated that the toxin specifically binds to *Torpedo* presynaptic membranes. The binding of crotoxin was inhibited, at least partially, by related PLA<sub>2</sub> neurotoxins but not by CB. Although CA alone does not bind, it competes efficiently with crotoxin. An analysis of the kinetics of crotoxin binding to *Torpedo* synaptosomal membranes further indicated that CA participates with CB to the formation of a ternary complex with the crotoxin binding site on presynaptic plasma membrane, preceding the release of CA.

MECHANISM OF ACTION OF  $\beta$ -BUNGAROTOXIN ( $\beta$ -BUTX), A  
PRESYNAPTICALLY ACTING PHOSPHOLIPASE A<sub>2</sub> (PLA<sub>2</sub>)  
NEUROTOXIN

P. Rosenberg, E.Ueno and R. Chapell

Dept. of Pharmaceutical Sciences, University of Connecticut,  
School of Pharmacy, Storrs, CT 06269, U.S.A.

$\beta$ -BUTX is a very potent PLA<sub>2</sub> neurotoxin which acts presynaptically to modify neurotransmitter release. In contrast, many other PLA<sub>2</sub> enzymes from snake venom are homologous to the PLA<sub>2</sub> chain of  $\beta$ -BUTX, have higher PLA<sub>2</sub> activity, and yet their pharmacological effects are rather weak and non-specific.  $\beta$ -BUTX initially binds to a specific receptor on the presynaptic terminal and after partially penetrating into the membrane causes an inhibition of a K<sup>+</sup> channel resulting in a transient stimulation of transmitter release. Subsequently there is an inhibition followed by block of release. Our results suggest that this is not due to an interference in the docking or fusion of the synaptic vesicles (SV) with the synaptic plasma membrane (SPM) since binding of isolated radioiodinated SV to SPM is unaffected. However, our results suggest that the accessibility of the SV to the SPM is decreased as a result of inhibition of phosphorylation of critical nerve terminal proteins, such as synapsin, GAP-43 and MARCKS. This inhibition does not appear to be due to a decrease in ATP levels, an increase in phosphatase activity, an effect of eicosanoids or an alteration in calmodulin levels. Using synthetic substrates we find that  $\beta$ -BUTX has no direct effect on the activity of protein kinases (PKA, PKC, CaMKII). We suggest therefore that  $\beta$ -BUTX, by an action on the membrane or directly on the kinase substrates, renders them less susceptible to phosphorylation. (Supported by NIH ROINS14521 to P.R.).

## SUBUNIT ASSOCIATIONS IN RATTLESNAKE PRESYNAPTIC NEUROTOXINS AND *PSEUDOCERASTES FIELDI* NEUROTOXIN.

Ivan I. Kaiser, Brian Francis, Jason Meng, and Corrine Seebart (Department of Molecular Biology, University of Wyoming, Laramie, WY 82071, U.S.A.)

Subunit associations of *P. fieldi* neurotoxin and rattlesnake presynaptic neurotoxins, as well as interactions of individual acidic and basic subunits from these heterodimeric complexes have been examined by gel-filtration and light scattering techniques. Substantial differences in elution volumes from gel-filtration columns can result from pH solvent changes, addition of  $\text{Ca}^{++}$ -ions, and anion differences, suggesting changes in subunit interactions. Light scattering measurements carried out in parallel however, suggest that in most cases elution changes are best explained by altered binding of proteins to the gel filtration matrix and not by changes in subunit association states. Subunit associations in each toxin have ionic and hydrophobic components. Intact Mojave toxin is highly resistant to trypsin digestion, but the basic subunit by itself is not. Initial cleavage of the basic subunit occurs at the end of the N-terminal helix (Arg-14) suggesting that this region is protected from trypsin digestion by association with the acidic subunit. Using these results, the primary sequence of each toxin's subunit, our knowledge of the structure of the pro-acidic crotoxin subunit, immunological results, and modeling of crotalid  $\text{PLA}_2$ s--based on the structure of *Crotalus atrox*  $\text{PLA}_2$ --we have proposed a model for rattlesnake heterodimeric subunit interactions. Surface regions of the acidic and basic subunits likely to be involved in subunit associations are identified. Since *P. fieldi* toxin basic subunit does not have the same hydrophobic and basic regions as crotoxin basic subunit, and *P. fieldi* toxin acidic subunit does not have the same hydrophobic and acidic regions as crotoxin acidic subunit, the two types of association probably have little in common. However, acidic subunit binding to part of the basic subunit interfacial binding surface may provide a similar mechanism for potentiation of toxicity. This exercise provides a working model for heterodimeric toxin structure. Supported in part by USAMRDC grant DAMD-17-92-J-2017 to I.I.K.

## Affinity Purification and Cloning of Taipoxin-binding Proteins

Anne K. Schlimgen, D Dodds, S.-Y. Lu, J.A. Helms and M.S. Perin

Division of Neuroscience Baylor College of Medicine; Houston, TX

We have identified, by affinity chromatography, 47 and 49 kDa binding proteins for the snake venom toxin taipoxin. The sequence of the 47 kDa protein (Neuronal Pentraxin or NP) is characteristic of a secreted protein and has homology to the acute phase proteins, serum amyloid P protein and C-reactive protein, of the pentraxin family. Northern analysis and in situ hybridization demonstrate high NP message levels in neurons of cerebellum, hippocampus and cerebral cortex. Because NP may be released synaptically and has homology to immune proteins potentially involved in uptake of lipidic, toxic or other antigenic material, we suggest that NP may be involved in a general uptake of synaptic macromolecules. The 49 kDa protein (Taipoxin-associated Calcium Binding Protein 49 or TCBP-49) is a luminal calcium binding protein that contains six EF hand calcium binding domains and the carboxyl-terminal sequence H-D-E-L, identical to the yeast ER retention signal. Message for this protein is present in brain, liver, muscle, heart, kidney and testis. Antibodies to this protein label reticular organelles of neurons and glia. The structure of NP and TCBP-49 and their localization to neurons raise the possibility that these proteins sequentially mediate the internalization and perhaps activation of taipoxin toxicity.

## MOLECULAR EVOLUTION OF PHOSPHOLIPASE A<sub>2</sub> TOXINS IN *VIPERA* *AMMODYTES*

F. Gubenšek, D. Kordiš

Department of Biochemistry and Molecular Biology, J. Stefan Institute, 61111  
Ljubljana, Slovenia

The abundance of isotoxins in snake venoms demonstrates an efficient evolutionary activity by which poisonous snakes acquired a number of new pharmacologically active proteins. In the type II phospholipase A<sub>2</sub> family of snake enzymes alone, more than ten such new properties were described including presynaptic neurotoxicity and myotoxicity. The ancestral PLA<sub>2</sub> gene apparently underwent several duplications which allowed mutations in some of the genes leading to new pharmacologically active isoforms. The sequences of the genes of non-toxic amodytin I<sub>2</sub>, of neurotoxic ammodytoxin C and of myotoxic ammodytin L were determined and compared with *Trimeresurus flavoviridis* and *Crotalus scutulatus scutulatus* PLA<sub>2</sub> genes. Structural composition of all three genes conforms with type II PLA<sub>2</sub> genes having 5 exons and 4 introns. The last three exons coding for mature proteins are much more mutated than introns where similarity between the three isoenzymes exceeds 93%. An exception is only a part of the 4th intron in ammodytin L and ammodytoxin C genes where an ART-2 retroposon was inserted, apparently before the two toxin genes duplicated and after the separation of the toxic and non-toxic PLA<sub>2</sub> genes. The phenomenon of highly conserved introns in snake toxins remains unexplained. The genes of mammalian type II PLA<sub>2</sub> enzymes had entirely different evolutionary pathway and show little similarity with snake enzymes. The structural organization and the intron phase is, however, conserved. Comparative analysis of the promoter region of type II PLA<sub>2</sub> genes revealed a high similarity in transcription factor binding sites in the proximal part of promoter region between bases -150 and -1 from the 1st exon. There is no doubt that the toxic enzymes appeared as a result of the positive Darwinian evolution of the non-toxic enzyme.

## Accelerated Evolution of Snake Venom Phospholipase A<sub>2</sub> Isozymes to Acquire Diverse Functions

<sup>1</sup>Tomohisa Ogawa, <sup>1</sup>Kin-ichi Nakashima, <sup>1</sup>Ikuo Nobuhisa, <sup>1</sup>Masanobu Deshimaru, <sup>1</sup>Yasuyuki Shimohigashi, <sup>2</sup>Yasuyuki Fukumaki, <sup>3</sup>Yoshiyuki Sakaki, <sup>3</sup>Shosaku Hattori, and <sup>1</sup>Motonori Ohno

<sup>1</sup>Dept. of Chemistry, Fac. of Science, and <sup>2</sup>Institute of Genetic Information, Kyushu University, Fukuoka 812 Institute of Medical Science, <sup>3</sup>the University of Tokyo, Tokyo 108, Japan

Snake venoms contain many Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) isozymes, which show a variety of physiological functions such as cardiotoxicity, neurotoxicity, myotoxicity, anticoagulant activity, and edema-inducing. The question has been raised how these PLA<sub>2</sub> isozymes with diverse physiological functions have evolved from ancestral PLA<sub>2</sub>. In the present study, we determined the nucleotide sequences of cDNAs and genes encoding *Trimeresurus flavoviridis* and *T. gramineus* snake venom PLA<sub>2</sub> isozymes. They revealed that the protein-coding regions are much more diversified than the 5' and 3' untranslated regions (UTRs) and the introns except for the signal peptide domain when compared between species and within species. To gain a further insight into this evolutionary phenomenon, the nucleotide sequences of the genes encoding *T. flavoviridis* and *T. gramineus* venom gland PLA<sub>2</sub> isozymes were analyzed by computing the numbers of nucleotide substitutions per synonymous site ( $K_S$ ) and per nonsynonymous site ( $K_A$ ) for the protein-coding regions and the numbers of nucleotide substitutions per site ( $K_N$ ) for the UTRs including introns. Furthermore, TBP (TATA box-binding protein) genes of *T. flavoviridis* and *T. gramineus* were analysed as the representative of nonvenomous genes and compared with PLA<sub>2</sub> isozyme genes. The data shows several characteristics. First, the  $K_N$  values for the 5' and 3' UTRs and introns of PLA<sub>2</sub> isozyme genes are approximately one-fourth of the  $K_S$  values of those genes and are at the same level as the  $K_N$  values of TBP genes, indicating that the protein-coding regions of PLA<sub>2</sub> isozyme genes are unusually variable. The  $K_A$  values were close to or larger than  $K_S$  values for relevant pairs of genes, indicating that the protein-coding regions of snake venom gland PLA<sub>2</sub> isozyme genes have evolved by accelerated evolution. Such novel evolutionary events of snake venom gland PLA<sub>2</sub> isozymes and their manifestation of diverse physiological activities enabled us to propose a working hypothesis that snake venom PLA<sub>2</sub> isozymes have been evolving under adaptive pressure to acquire new physiological activities.



DO WE KNOW THE COMPLETE SEQUENCE OF  
METALLOPROTEINASE AND NONENZYMATIC PLATELET  
AGGREGATION INHIBITOR (DISINTEGRIN) PRECURSOR PROTEINS?

R. Manjunatha Kini  
Bioscience Centre, Faculty of Science  
National University of Singapore, Lower Kent Ridge  
Road, Singapore 0511

Recent evidence indicates that metalloproteinases and disintegrins, nonenzymatic inhibitors of platelet aggregation, are derived by proteolysis from common precursors. Although proteins and polypeptides with various domain structures have been identified, proteins containing proprotein domains or the complete mature precursors have not been isolated so far. This prompted a closer examination of the putative start codon, signal peptide and the segment upstream of these regions. Firstly, the 5'-region upstream of the putative start ATG codon can code for protein in the same reading frame. Secondly, the putative signal peptide does not contain any positively charged residues. Thirdly, the signal processing site does not follow (-3, -1) rule. Finally, some evidence indicates that C-type lectin-related proteins are derived from the upstream 5'-region precursors. Thus, a critical evaluation of sequence information of these precursors indicates that the putative signal peptide identified in these precursors may be an internal hydrophobic segment within the precursor. Therefore the available sequence data of the precursors appears to be incomplete.

I thank Dr. Michael J. Holmes, Bioscience Centre and Dr. Ruowen Ge, Department of Zoology for their critical comments. This work is supported by a financial grant from the Economic Development Board of Singapore.

**Snake Venom Metalloproteinases: Structure, Function and Relationship to the Mammalian ADAMs Family of Proteins.** <sup>1</sup>Jay W. Fox, <sup>1</sup>Li-Guo Jia, <sup>2</sup>Jon B. Bjarnason and <sup>1</sup>Ken-Ichi Shimokawa. University of Virginia Health Sciences Center, Charlottesville, VA and <sup>2</sup>University of Iceland, Reykjavik, Iceland.

A large number of zinc metalloproteinases of varying molecular weights and biological functions has been isolated from crotalid and viperid snake venoms. Recent studies have illustrated that based on domain structure there are four size classes (P-I to IV) of these proteinases. They are synthesized as zymogens which become processed in the crude venom to the active protein form. The mechanism of latency appears to be homologous to that of matrix metalloproteinases in that a "cysteine switch" is likely involved. The mechanism of activation of these proteinases is not clear, however autoactivation has been implicated. The proteinase domains of these proteins have either of two general forms: a two or three disulfide bond structure. Crystallographic and molecular modeling studies suggest that the tertiary structures of the two forms are similar. The larger venom metalloproteinases have additional domains carboxy to the zinc binding domain. In some instances (class P-II) these domains are further processed to give rise to free domains such as with the disintegrins. Class P-III has disintegrin-like and cystine rich domains. Class P-IV is similar to P-III but also has additional lectin-like domains. These non-enzymatic domains likely modulate the overall biological activity of the proteinases. Recently, homologs of the venom P-III structures have been identified in a variety of mammalian tissues. These proteins have been termed ADAMs for A Disintegrin-like And Metalloproteinase containing protein. The mammalian proteins are integral membrane proteins which have pre-, pro-, proteinase, and cystine rich domains which are homologous to the venom proteins. The current understanding of the functional properties of the snake venom metalloproteinases have impacted the studies of the mammalian homologs and may give insight into their biological activities.

## CHARACTERIZATION OF THE MAJOR METALLOPROTEASE ISOLATED FROM THE VENOM OF *CROTALUS VIRIDIS OREGANUS*

Stephen P. Mackessy, Dept. of Biological Sciences, University of Northern  
Colorado, Greeley, CO, 80639 USA.

Email: smackess@goldng8.univnorthco.edu

Rattlesnake venoms typically contain several different metalloproteases, some of which are hemorrhagic toxins. Metalloproteases are likely responsible for the often severe necrotic changes in tissues following envenomation, and these prominent components are important (to the snake) to the predigestive role of venoms. Venom of the northern Pacific rattlesnake (*C. v. oreganus*) contains at least 4 distinct metalloproteases, and the dominant protease has been isolated and characterized. It is a single polypeptide chain toxin with a molecular weight of 61,000, is an acidic protein and has a pH optimum of ~9.0. It catalyzes the hydrolysis of several protein substrates including casein, and it is inhibited by metal chelators such as EDTA and 1,10-phenanthroline but not by serine protease inhibitors such as PMSF. Caseinolytic activity is not significantly inhibited by citrate (at pH 9.0) at levels up to 2 mM; at 100 mM citrate, >65% activity is retained. It is partially inhibited by nanomolar concentrations of ATP, but higher amounts (micromolar) do not result in further inhibition of activity. The protease shows fibrinolytic and fibrinogenolytic activity, but is only weakly hemorrhagic in rats. When stored lyophilized or in solution it undergoes autolytic degradation. This protease or a homolog appears to be present in venoms from several rattlesnake species but it is not present in venoms of juvenile *C. v. oreganus*. The presence of this component in venoms from adult rattlesnakes is responsible for the age-related increase in metalloprotease activity of the crude venom.

## POTASSIUM CHANNEL BLOCKING ACTIONS OF DENDROTOXINS

A.L. Harvey

Department of Physiology and Pharmacology, Strathclyde Institute for Drug  
Research, Strathclyde University, Glasgow G1 1XW, UK

Dendrotoxins are 57-60 residue proteins isolated from venoms of mamba snakes, *Dendroaspis angusticeps*, *D. polylepis* and *D. viridis*. They are related to Kunitz-type inhibitors of serine proteases. Six natural variants have been sequenced: two isoforms of  $\alpha$ -dendrotoxin and  $\delta$ -dendrotoxin from *D. angusticeps*, toxin I and toxin K from *D. polylepis*, and Dv 14 from *D. viridis*. Two related toxins,  $\beta$ - and  $\gamma$ -dendrotoxin, from *D. angusticeps* have been partially sequenced. The dendrotoxins were discovered because of their ability to facilitate the release of acetylcholine at the neuromuscular junction, which is a consequence of their ability to block some voltage-dependent  $K^+$  channels in nerve endings. Dendrotoxins block some neuronal  $K^+$  channels but not others, and often induce repetitive action potentials. As  $\alpha$ - and  $\delta$ - dendrotoxins appear to block different  $K^+$  currents in synaptosomes from those blocked by  $\beta$ - and  $\gamma$ - dendrotoxins, it is likely that different subtypes of voltage-dependent  $K^+$  channels will have different sensitivities to individual dendrotoxins. Analysis of structure-activity relationships of dendrotoxins should reveal information about the molecular recognition sites on different subtypes of  $K^+$  channels. Comparison of the primary sequences of dendrotoxins and inactive homologues highlights conserved Lys residues in the dendrotoxins. Acetylation of these residues in toxin I from *D. polylepis* was followed by assessment of binding affinity to rat brain synaptosomal membranes and facilitatory activity on chick biventer cervicis nerve-muscle preparations (Harvey & Karisson, to be published). Four of the mono-modified derivatives were similar to native toxin in activity, two had lost about 10-30 fold activity, and one had lost about 200 fold activity. In contrast, a version of  $\alpha$ -dendrotoxin in which the lysine triplet 28-30 was mutated to Ala-Ala-Gly lost less than 10 fold activity (Danse *et al.*, 1994, FEBS Lett. 356, 153). Hence, some Lys residues may be important for activity, but they are not at the previously highlighted "lysine site".

SITE-DIRECTED MUTANTS OF DENDROTOXIN K REVEAL THAT  
POSITIVELY CHARGED RESIDUES FROM TWO STRUCTURAL DOMAINS  
OF THE PROTEIN ARE IMPORTANT FOR K<sup>+</sup> CHANNEL INTERACTION

Paul Reid<sup>1</sup>, Fancheng Wang<sup>2</sup>, Mark Olson<sup>1</sup>, James Schmidt<sup>1</sup>,  
Dave Parcej<sup>2</sup>, Oliver Dolly<sup>2</sup> and Leonard Smith<sup>1</sup>

<sup>1</sup>Toxinology Division, USAMRIID, Fort Detrick, MD 21702, USA.

<sup>2</sup>Department of Biochemistry, Imperial College, London, SW7 2AY, UK.

Dendrotoxin K (DTX<sub>K</sub>) is a single-chain, basic polypeptide of 57 residues isolated from the venom of the black mamba. DTX<sub>K</sub>, like other dendrotoxin homologues, is a potent inhibitor of voltage-sensitive K<sup>+</sup> channels. The NMR solution structure of DTX<sub>K</sub> indicates that the protein has a 3<sub>10</sub>-helix of residues 3 to 7, a distorted β-hairpin of residues 18 to 35, and an α-helix of residues 47 to 56. While the tertiary structure of DTX<sub>K</sub> is known, a detailed molecular understanding of the functional role of individual amino acid residues remains rudimentary. As an essential first step in elucidating structure-activity relationships, we cloned the cDNA encoding DTX<sub>K</sub> and expressed it in *E. coli* as a fusion protein with maltose-binding protein (MBP). The DTX<sub>K</sub>-MBP fusion protein was affinity purified on an amylose resin and DTX<sub>K</sub> cleaved from the MBP chaperone with Factor X. DTX<sub>K</sub> was purified to homogeneity by using reverse-phase chromatography. The recombinant DTX<sub>K</sub> was identical to native DTX<sub>K</sub> in N-terminal sequence, chromatographic behavior, convulsive-inducing activity, and binding to voltage-activated K<sup>+</sup> channels from bovine and rat brain synaptic plasma membranes. Using the cDNA construct, we generated a panel of site-directed DTX<sub>K</sub> mutants and determined their affinities for voltage-dependent K<sup>+</sup> channels on the basis of competitive-binding assays by using rat brain synaptic plasma membranes. These data show that residues analyzed in a 'random coil' region of DTX<sub>K</sub> (region of the antiprotease loop in BPTI) and in the α-helix region do not contribute significantly to the high-affinity binding sites on voltage-dependent K<sup>+</sup> channels. In contrast, when lysine residue 26 located in the β-turn or lysine residue 3 situated in the 3<sub>10</sub>-helix were mutated, the high-affinity binding sites to K<sup>+</sup> channels were notably reduced. These data are consistent with the hypothesis that the binding between DTX<sub>K</sub> and the K<sup>+</sup> channel is an electrostatic interaction, since it was recently shown that the α-DTX binding site on rat brain K<sup>+</sup> channel is located in an extracellular domain, which includes an essential negatively charged residue. We conclude that positively charged residues located in the β-turn and the 3<sub>10</sub>-helix of DTX<sub>K</sub> provide surface complementarity with negatively charged residues found in the binding domain of the K<sup>+</sup> channel.

## RECOMBINANT $\alpha$ -BUNGAROTOXIN CONSTRUCTS AND MUTANT ANALYSIS

E. Hawrot, Q-L. Shi, and J.A. Rosenthal

Department of Molecular Pharmacology and Biotechnology, Division of Biology  
and Medicine, Brown University, Providence, RI 02912.

We have been able to express and recover from *E. coli* a recombinantly produced  $\alpha$ -Bungarotoxin (Bgtx) designed as a fusion protein with a highly soluble T7 coat protein encoded by gene 9 of bacteriophage T7. After release of the recombinant Bgtx by proteolytic cleavage, the isolated recombinant Bgtx was found to contain ten additional residues attached to the N-terminus. The binding activity of this modified Bgtx was within a factor of two of that obtained with authentic Bgtx indicating that the N-terminus is not an important region in receptor recognition. Mutation of the conserved Asp at position 30 to an isosteric Ala produced no reduction in the activity of the recombinant Bgtx suggesting similarly that the carboxylate of Asp30 is not required for binding. We have now inserted a seven amino acid histidine "tag" N-terminal to the Bgtx sequence and C-terminal to an engineered thrombin protease site. Cleavage of the affinity-purified Histag-fusion protein, results in a ~9 kD protein. A final purification step using cation exchange chromatography separates fully active material (~10-50% of the total) from fractions with reduced activity. The fully active material is indistinguishable from native toxin under the solid-phase, binding competition assay conditions used. These are based on electric organ membranes from *Torpedo californica* as the source of native receptor. We now produce ~600  $\mu$ g of active toxin/L of starting culture, a 25% increase in active toxin produced in comparison to the non-Histag protein. We further report the purification of two additional mutants of Bgtx, an Arg36Ala substitution and a truncation mutant in which the carboxy-terminal 7 residues have been deleted. Substitution of Arg36 with Ala results in a ~20-fold reduction in binding activity. We observe an 11-fold reduction in binding affinity in the truncated form of Bgtx.

## ON THE RECOGNITION SITE OF AN ANIMAL TOXIN, AS STUDIED BY SITE-DIRECTED MUTAGENESIS.

Denis Servent, Valérie Winckler-Dietrich, Cécile Fromen,  
Frédéric Ducancel, Jean-Claude Boulain & André Ménez.

Understanding of the molecular mechanisms associated with the toxic action of an animal toxin requires, at least in a first step, elucidation of the site by which the toxin recognizes its molecular target. In this respect, we are currently investigating the site by which erabutoxin a (Ea), a curaremimetic toxin from a sea snake venom, recognizes the nicotinic acetylcholine receptor (AcChoR) from fish, i.e. *Torpedo marmorata* with high affinity ( $K_d = 70$  pM) and great specificity.

We cloned the cDNA encoding Ea, fused it to the gene encoding the staphylococcal protein A, expressed the fused gene in *E. coli* and cleaved the resulting fusion protein by CNBr. The recombinant Ea was functionally and structurally indistinguishable from the snake toxin. We generated a large series of toxin mutants and determined their affinities for AcChoR, on the basis of competition experiments. The residues whose mutation substantially decreased Ea affinity, defined a functionally important area which includes not only residues that are conserved in most curaremimetic toxins but intriguingly also, residues that are variable from one toxin to another. The functionality of variable residues suggests that the recognition site identified in Ea is different in other curaremimetic toxins and that it might have undergone an adaptative evolution for AcChoR from fish, a well-known prey of sea snakes.

It is known that an animal toxin can exhibit quite different binding affinities toward two similar receptors from distinct origins. In this respect, we found that Ea has a low affinity ( $K_p = 25$   $\mu$ M) toward the chimaeric  $\alpha 7$ -V201-5HT3, a homooligomeric receptor which possesses the same ligand profile as the wild-type neuronal AcChoR  $\alpha 7$  from chick brain. To delineate the site by which Ea recognizes  $\alpha 7$ -V201-5HT3 and to understand why this toxin has such a low affinity for this receptor, we investigated the binding affinities of some selected mutants on the basis of competition analyses using  $\alpha 7$ -V201-5HT3 expressed in transiently-transfected HEK cells. The results of this preliminary investigation will be presented and discussed in the frame of possible evolutionary mechanisms of the recognition site of an animal toxin.

THE BINDING SITE FOR  $\kappa$ -NEUROTOXINS  
ON NEURONAL NICOTINIC RECEPTORS PROBED WITH  
SNAKE-VENOM-DERIVED AND RECOMBINANT TOXINS

<sup>1</sup>V.A. Chiappinelli, <sup>1</sup>W.R. Weaver, <sup>2</sup>B.M. Conti-Fine,  
<sup>3</sup>J.J. Fiordalisi and <sup>3</sup>G.A. Grant

<sup>1</sup>Saint Louis University, St. Louis MO 63104 USA,

<sup>2</sup>University of Minnesota, St. Paul MN 55108 USA,

<sup>3</sup>Washington University, St. Louis MO 63110 USA

The  $\kappa$ -neurotoxins are useful probes for distinguishing neuronal nicotinic acetylcholine receptors because they are potent antagonists at only a subgroup of these receptors, all of which contain at least one  $\alpha 3$  subunit. Four of these highly homologous, 66-amino acid long peptides have been purified from the venoms of *Bungarus multicinctus* ( $\kappa$ -bungarotoxin ( $\kappa$ BgT),  $\kappa_2$ BgT,  $\kappa_3$ BgT) and *B. flaviceps* ( $\kappa$ -flavitoxin ( $\kappa$ FvT)). We have taken two different approaches to examine the binding of these toxins to neuronal nicotinic receptors.

In the first approach, we radioiodinated venom-derived  $\kappa$ FvT and  $\kappa$ BgT and measured the specific binding of these toxins to overlapping synthetic peptides (15-21 amino acids in length) that were prepared based on the known sequence of the nicotinic receptor  $\alpha 3$  subunit. At least two main regions of interaction between the toxins and the receptor subunit were identified, both of which lie in the N-terminal region of the subunit that is exposed to the extracellular space.

In the second approach, we examined the importance of certain amino acids within the toxins for the maintenance of high affinity binding to  $\alpha 3$ -containing receptors in autonomic ganglia. This was done using site-directed mutants of  $\kappa$ BgT produced by an *Escherichia coli* expression system. Both Arg-40 and Pro-42 were found to be important for this interaction.

The results confirm that  $\kappa$ -neurotoxins bind to the  $\alpha 3$  subunit, and that site-directed mutagenesis of recombinant  $\kappa$ BgT is an important approach for the study of structure/function relationships between  $\kappa$ BgT and nicotinic receptors. NIH Support: NS17574 (V.A.C.), DA05695 (B.M.C.) and GM50624 (G.A.G.).



ANTI-MUSCARINIC TOXINS FROM *DENDROASPIS ANGUSTICEPS*  
LT Potter, JM Carsi-Gabrenas, SL Purkerson, MP Santiago, WL Strauss  
and HH Valentine, Department of Molecular and Cellular Pharmacology, U.  
of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101, U.S.A.

After Karlsson's laboratory demonstrated in 1988 that the venom of the green mamba contains two toxins that block the binding of  $^3\text{H}$ -QNB to rat cortical muscarinic receptors, this laboratory began to test components of this venom for the specific blockade of pure cloned m1-m5 muscarinic receptors. It is now evident from conventional purification and molecular cloning techniques that the venom contains more than ten toxins that affect m1, m2, m3 and m4 receptors. So far no toxins show major anti-m5 activity. The most active and specific anti-m1 toxin is m1-toxin and the most active and specific anti-m4 toxin is m4-toxin (Potter et al, *Life Sciences* 52, 433, 1993; Max et al, *J. Neurosci.* 13, 4293, 1993, *J. Pharmacol.* 26, 7480, 1993, *Mol. Pharmacol.* 44, 1171, 1993, *Neurosci. Abstr.* 19, 462, 1993; Potter and Purkerson, In *Molecular and Cellular Mechanisms of Neostriatal Function*, Landes Co, NY, 1995). New two-step procedures have been devised to purify each toxin. All are homologous to short chain anti-nicotinic toxins and have four disulfide bonds. m1-Toxin binds to the extracellular face of m1 receptors in 30 seconds even at subsaturating concentrations and at 4°C, whether or not these receptors are occupied by an antagonist, and the toxin remains bound at 37°C with a  $T_{1/2}$  of about 6 hours. At high concentrations m1-toxin can bind to m4 receptors, but it dissociates so rapidly that it is effectively specific for m1 sites. In comparison, m4-toxin binds reversibly to m4 receptors with an affinity ( $K_d \approx 0.95$  nM) 102-fold higher than to m1 receptors, and not to m2, m3 or m5 sites. Both toxins are excellent functional antagonists, remain selective in solution and retain high activity when labeled. These toxins are now being used for specific and coordinated cytological, electrophysiological, biochemical and behavioral studies of m1 and m4 receptors at central and peripheral cholinergic synapses.

Atroxase: A Fibrinolytic Enzyme Isolated from the Venom of Western Diamondback Rattlesnake: Isolation, Characterization and Cloning.

Brenda J. Baker<sup>1</sup> and Anthony T. Tu<sup>2</sup>

<sup>1</sup>Department of Chemistry, University of Southern Colorado, Pueblo, CO 81001

<sup>2</sup>Department of Biochemistry & Molecular Biology, Colorado State University, Fort Collins, CO 80523-1870

Snake venom is a mixture of toxic and nontoxic proteins. Atroxase, a nontoxic protein isolated from the venom of Crotalus atrox (western diamondback rattlesnake), dissolves artificially induced thrombolytic blood clots in vitro and in vivo, with no apparent toxic effects. In an effort to determine the primary structure of atroxase, a cDNA library, prepared from the activated venom glands of one specimen of Crotalus atrox, was probed for cDNA encoding the atroxase protein. Degenerate synthetic oligonucleotide probes were designed based on the known partial amino acid sequence of atroxase. Using these degenerate probes, gene fragments were isolated from the cDNA library via the polymerase chain reaction. Two fragments, 200 and 400 bases respectively, were isolated on PEG agarose, transformed into pBluescript vectors, and subsequently sequenced by the Sanger dideoxy sequencing method. The two fragments, when translated, are homologous to the previously deduced partial amino acid sequence of atroxase. These PCR-derived fragments were radiolabeled with <sup>32</sup>P and used to specifically probe the original cDNA library via colony hybridization. Positive cDNA clones were isolated and sequenced resulting in a truncated cDNA clone of the atroxase gene. The final nucleotide sequence shows high homology to previously described primary structures of nonhemorrhagic fibrinolytic proteases isolated from snake venom. The goal is to express the biologically active protein for further structure function studies on the thrombolytic activity of atroxase.

STUDIES ON THE MECHANISM OF ACTION OF MYOTOXINS ISOLATED FROM  
BOTHROPS SNAKE VENOMS

J.M. Gutiérrez and B. Lomonte

Instituto Clodomiro Picado, Facultad de Microbiología,  
Universidad de Costa Rica, San José, Costa Rica

Several myotoxins have been isolated from Bothrops spp snake venoms. All of them are group II basic phospholipases A<sub>2</sub>, although some lack enzymatic activity (i.e. Lys-49 variants). Myotoxins are quantitatively important venom components in some Bothrops species. Intramuscular injection of these myotoxins leads to a drastic series of rapid degenerative events, probably initiated at the plasma membrane level, which culminate with a selective skeletal muscle necrosis. This in vivo specificity contrasts with the ability of myotoxins to affect many types of cells in culture. Muscle damage, as well as cytolysis and liposome disruption, occur in conditions where phospholipase activity is inhibited, although enzymatic activity might enhance myotoxin actions. A membrane receptor for Bothrops myotoxins has not been identified yet. A working hypothesis on the mechanism of action has been proposed. Current evidence suggests that these toxins interact with biological membranes via a molecular region distinct from the catalytic site. The active region is likely to be formed by a combination of basic and hydrophobic amino acid residues near the C-terminus of the protein, that allow an electrostatic binding interaction and a bilayer penetration. In addition, acylation of myotoxins may enhance their membrane-disrupting effects. These events lead to membrane destabilization and loss of selective permeability to ions such as calcium, with the consequent deleterious effects on muscle cells.

These studies have been supported by Universidad de Costa Rica, International Foundation for Science and Third World Academy of Sciences.

## SIMILARITIES AND DIFFERENCES IN MECHANISMS OF CARDIOTOXINS, MELITTIN AND OTHER MYOTOXINS

J.E. Fletcher, Q-H. Gong and M-S. Jiang

Medical College of PA and Hahnemann Univ., Philadelphia, PA U.S.A.

The cardiotoxins and melittin share a wide variety of actions and sorting out cause-effect relationships has been difficult (Toxicon **31** 669-695, 1993). In addition to their cytolytic action, both cardiotoxin and melittin are myotoxic. Thus, we compared specific actions of these cytolytic peptides to other myotoxic agents. There are two basic types of myonecrosis. The noncytolytic peptides, such as myotoxin  $\alpha$  and crotamine cause relatively little change in gross morphology over the first 12 hrs and have no effect on the plasma membrane after 48 hr when damage of cell membranes is more obvious. In contrast, the phospholipase  $A_2$  (PLA $_2$ ) myotoxins and cytolytic peptide myotoxins rapidly lyse the plasma membrane and cause gross necrosis. The noncytolytic peptides do have some rapid effects on skeletal muscle, such as the induction of contractures, which occur in seconds to minutes. All of the myotoxins tested (cardiotoxin, melittin, crotamine, myotoxin  $\alpha$ , bothropstoxin) decrease net  $Ca^{2+}$  uptake in isolated sarcoplasmic reticulum preparations and this action appears to be due to stimulation of one or more  $Ca^{2+}$  release processes. Several studies also suggest that the  $Na^+$  channel is a target common to all of these toxins. One action that separates the PLA $_2$  myotoxins and cytolytic peptide myotoxins from the noncytolytic peptides, is the production of fatty acids. In the case of the PLA $_2$  myotoxins, fatty acid production is the direct result of the toxin PLA $_2$  activity; whereas with the cytolytic peptides the fatty acids are derived from tissue phospholipase C and, possibly, tissue PLA $_2$ . Despite very different structures, the myotoxic agents share many mechanisms of action. Lipid metabolism may be an important factor increasing the rapidity and extent of myotoxicity.

## MYOTOXICITY OF VENOMS AND TOXINS

P GOPALAKRISHNAKONE

Venom & Toxin Research Group, National University of Singapore,  
Singapore 0511

The term "Myotoxicity" is used in a wider sense when a venom or toxin acts on skeletal muscle and causing changes in the muscle as shown either by biochemical, pharmacological or morphological evidence. There are two types of PLA<sub>2</sub> toxins which cause muscle damage, crotoxin, taipoxin, notexin and mojave toxin produce muscle degeneration followed by regeneration when injected locally. Whereas PLA<sub>2</sub> from Enhydrina schistosa and PLA<sub>2</sub>\* from Australian elapids of the species Pseudechis, produce systemic myotoxicity, characterized by myoglobinuria and generalized muscle damage. Muscle damage could be observed by light and electron microscopic methods. The changes include dilatation of sarcoplasmic reticulum, vacuolation, oedema within first four hours of injection of toxin, followed by disruption and hypercontraction of the fibres with inflammatory changes by about 6 hr. The inflammatory reaction characterized by infiltration by phagocytic cells was maximally seen between 12-24 hr. Evidence of regeneration starts by 36 hr and complete regeneration was seen by 7-10 days. The mechanism/s which these toxins cause muscle damage is not fully understood. However, the disruption of sarcolemmal membrane, increase in influx of Ca<sup>2+</sup> into sarcoplasm as well as Ca<sup>2+</sup> activated neutral proteases have a definitive role in this process. The possible mechanism/s for muscle degeneration as well as structure function activity of the PLA<sub>2</sub> myotoxins in reference to enzymatic activity, neurotoxicity and myotoxicity will also be discussed. Scorpion venoms which cause myodegeneration also will be presented for the first time.

## cDNA CLONING AND PROTEIN SEQUENCE ANALYSIS OF A K49-PLA2 MYOTOXIN: A HIGHLY CONSERVED CLASS OF PROTEINS

Heloisa S. Selistre de Araujo<sup>1,3</sup>, Steven P. White<sup>2</sup> and Charlotte L. Ownby<sup>1</sup>

<sup>1</sup>Dept of Physiological Sciences and <sup>2</sup>Dept of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078, USA, <sup>3</sup>Dept de Ciências Fisiológicas, Universidade Federal de São Carlos, São Carlos, SP 13565, Brazil

A few catalytically inactive snake venom PLA2 myotoxins have been described, indicating that the PLA2 activity is not necessary for induction of myotoxicity. However, the myotoxic site in these molecules is still unknown. To aid the understanding of the complex structure-function relationship of the myotoxins, we have cloned and sequenced the cDNA for ACL myotoxin from an *Agkistrodon contortrix laticinctus* venom gland cDNA library. The library was made in the UNIZAP<sup>TM</sup> vector and screened with a degenerate oligonucleotide probe which was designed from the N-terminal sequence of the purified ACL myotoxin. One of the isolated clones (ACLPREMT1) is 734 bp in length and has an open reading frame of 414 bp. It codes for a K49 PLA2 with 121 amino acid residues of which the first 20 match exactly the N-terminal sequence of the purified ACL myotoxin. ACLPREMT1 has a domain organization that is very conserved in the type II PLA2 gene family in Viperidae snakes. Comparison of the predicted sequence of ACL myotoxin and other K49 and D49 PLA2 proteins shows that K49 PLA2 myotoxins have several very conserved residues not present in D49 enzymes. Also, sequence comparison and molecular modeling of ACL myotoxin show the presence of at least two different molecular sites in type II PLA2s. These results may help explain the difference in activities between distinct classes of PLA2s.

H.S.S.A. has a fellowship from CNPq, Brazil

## PARTIAL BIOCHEMICAL CHARACTERIZATION OF A MYOTOXIN FROM THE DUVERNOY'S SECRETION OF *PHILODRYAS OLFERSII*.

J. Prado-Franceschi<sup>1</sup>, S. Hyslop<sup>1</sup>, J.C. Cogo<sup>1</sup>, A.L. Andrade<sup>1</sup>, A.P. Reichl<sup>3</sup>, M.T. Assakura<sup>3</sup>, M.A. Cruz-Hofling<sup>2</sup>, and L. Rodrigues-Simioni<sup>1</sup>.

Departments of <sup>1</sup>Pharmacology and <sup>2</sup>Histology, UNICAMP, Campinas, SP, Brazil and <sup>3</sup>Laboratory of Biochemistry and Biophysics, Instituto Butantan, São Paulo, SP, Brazil.

The Duvernoy's secretion of the xenodontine colubrid *Philodryas olfersii* (green snake) possesses a variety of biological activities including fibrin(ogen)olytic, hemorrhagic and edematogenic actions [Assakura *et al*, *Toxicon* 30:427 (1992); 32:819 (1994)]. In the present work, the protein responsible for the myotoxic activity previously observed in the above secretion was purified to homogeneity by chromatography on Sephadex G-100 SF. In SDS-PAGE, the toxin migrates as a single band with a MW of 20,000. The myotoxin contains 182 amino acid residues (approx. 20% acidic) and has a blocked N-terminal amino acid. The pI of the protein is 4.8. *In vitro*, the myotoxin stimulates the release of creatine kinase from the chick *biventer cervicis* preparation in a manner similar to the crude Duvernoy's secretion. Histological examination showed that the myotoxin causes severe muscle necrosis involving disruption of the skeletal muscle cells with a total or partial loss of the transverse striations. These morphological changes are therefore similar to those observed with the crude secretion.

Financial support: FAPESP, FAEP/UNICAMP

## ERINACIN, AN ANTIHAEMORRHAGIC FACTOR FROM THE EUROPEAN HEDGEHOG, *ERINACEUS EUROPAEUS*

<sup>1</sup>D. Mebs, <sup>2</sup>T. Omori-Satoh, <sup>2</sup>Y. Yamakawa and <sup>2</sup>Y. Nagaoka

<sup>1</sup>Zentrum der Rechtsmedizin, University of Frankfurt, Frankfurt, Germany;

<sup>2</sup>National Institute of Health, Tokyo, Japan.

An antihaemorrhagic factor named erinacin was purified from the muscle extract of the European hedgehog, *Erinaceus europaeus*, by ammonium sulfate precipitation followed by various steps of ion-exchange (DEAE-cellulose) and absorption chromatography (hydroxyapatite), and gel filtration (cellofine gel). A 630-fold purification was achieved for erinacin with an overall yield of 19% of antihaemorrhagic activity. The protein effectively inhibits the activity of haemorrhagins from the venom of *Bothrops jararaca* and of *Bitis arietans*, as well as the enzymatic activity of trypsin (TAME as substrate) and of chymotrypsin (BTee as substrate). SDS-PAGE of erinacin revealed the presence of two subunits: one with an apparent mol. weight of 35kD and intramolecular SS-bridges and a second with a mol. weight of 39kD without disulfides. Dissociation of erinacin into the two subunits resulted in complete inactivation of its antihaemorrhagic activity.



**Bacterial Enterotoxins Affecting the Colon**  
**Tracy D. Wilkins**  
Center for Biotechnology, Virginia Tech

The colon is one of the most anaerobic habitats on earth. The normal colon is protected from pathogens by a high density of bacterial specialists. The use of broad-spectrum antibiotics in high dosage eliminates this protection, and causes diarrhea and colitis among patients being treated for other ailments.

*Clostridium difficile* is the best known cause of antibiotic associated diarrhea and colitis, but it is not the only cause. This organism produces two large toxins which alter the intracellular protein "rho" to cause disorganization of the cytoskeleton. This results in loss of function by the cells with resulting degradation of the intestinal epithelium. The enterotoxin (Toxin A) binds to cell surface carbohydrates in the colon, enters the epithelial cells and causes the diarrhea. The second toxin (Toxin B) binds to the non-epithelial cells which become exposed - resulting in colitis. Patients in the United States spend over \$200 million a year on diagnostic tests alone for these toxins. The total hospital bill is close to a billion dollars a year.

*Clostridium perfringens* enterotoxin, which causes food poisoning, also can cause antibiotic-induced diarrhea. This toxin binds to epithelial cells in the colon and alters their permeability. New tests for this toxin are showing us that the disease is much more common than previously recognized. It may turn out to rival *C. difficile*

*Bacteroides fragilis* enterotoxin causes diarrhea in a novel way - it produces a metalloprotease that degrades the proteins in the tight junctions between epithelial cells. The effect is like removing the mortar between the bricks in a wall. Diarrhea ensues from the break in permeability; bacteria and other toxins then are allowed access to the intracellular spaces. Toxigenic strains of *B. fragilis* are a normal part of the colonic flora of some people and animals - so degradation may be occurring continuously in some individuals. I believe that this could lead to increased risk of carcinoma of the colon. All of these toxins may act in synergy in patients with various types of colonic disease.

Tox 2 from *B. fragilis* has a 40% homology to Mozambique cobra cytotoxin. Tracy has cloned the gene encoding Tox 2. It is a zinc metalloproteinase (~20,000 MW).

**Overview of *Clostridium perfringens* Enterotoxin. Bruce A. McClane. Univ. of Pittsburgh Sch. Med., Pittsburgh, Pa 15261**

*C. perfringens* enterotoxin (CPE) is known to cause the symptoms associated with *C. perfringens* type A food poisoning; this toxin may also be involved in other gastrointestinal illnesses of humans and domestic animals. Recent studies indicate that CPE's pathophysiologic effects result from an apparently unique series of four molecular events which occur early in CPE action. These events appear to proceed as follows: i) CPE binds to an ~50 kDa receptor protein to form a small complex (90 kDa) in mammalian plasma membranes, ii) small complex then undergoes a physical (probably conformational) change, iii) this "physically-changed" small complex interacts with a 70 kDa membrane protein to form a large (160 kDa) complex in membranes and iv) a large complex forms and induces breakdown of the normal permeability properties of the plasma membrane for small molecules of <200 Daltons. These permeability changes eventually cause cell death. Studies on CPE structure/function relationships have localized a receptor-binding region to the extreme C-terminus of this 35 kDa single polypeptide toxin; sequences in the N-terminal half of CPE appear to be required for steps ii-iv in CPE action (described above). CPE is normally expressed only during the sporulation of *C. perfringens*. Recent studies have shown that when naturally *cpe*-negative *C. perfringens* isolates are transformed with the *cpe* gene, they can express CPE in a sporulation-dependent manner. This result suggests that CPE expression may involve a common regulatory factor which also regulates other sporulation-dependent gene expression in *C. perfringens*.

## THE STRUCTURE AND MECHANISM OF ACTION OF BOTULINUM TOXIN

Nabil Bakry, Julie Coffield, Andrew B. Maksymowych,  
Ren-de Zhang and Lance L. Simpson  
Division of Environmental Medicine and Toxicology,  
Department of Medicine, Jefferson Medical College,  
Philadelphia, PA 19107

Botulinum neurotoxin exists in seven different serotypes (A to G) that have similar macrostructures and mechanisms of action. Each of the serotypes acts preferentially on cholinergic nerve endings to block the release of acetylcholine. Studies on laboratory animal tissues indicate that blockade of exocytosis involves a progression of four steps, as follows: 1) binding to the plasma membrane of cholinergic nerve endings, 2) internalization by receptor-mediated endocytosis, 3) entry into the cytosol by pH-induced translocation, and 4) intracellular poisoning by a metalloendoprotease action that produces cleavage of peptides necessary for transmitter release (synaptobrevin, SNAP-25 and syntaxin). Recently, the mechanism of toxin action has been studied on isolated human tissues. This work has included electrophysiological studies of toxin action on excised neuromuscular junctions (pyramidalis nerve-muscle preparation), ligand binding studies of toxin association with nerve membranes, and molecular biology experiments to clone and sequence human genes that encode toxin substrates. The results of this work indicate that the human nervous system possesses substrates for serotypes A (SNAP-25), B (synaptobrevin) and C (syntaxin). Cloning and sequencing of human syntaxin revealed substantial homology with rat syntaxin A (~98%) and syntaxin B (~89%). Standard electrophysiological techniques were used to examine the sensitivity of the pyramidalis muscle to toxin-induced blockade. At a toxin concentration of  $10^{-8}$  M, spontaneous and evoked endplate activity was decreased ( $\geq 90\%$ ) within 90 min by serotype A and 60 min by serotype B. In addition to experiments on tissues from patients without apparent neurologic disorders, studies were also performed on tissues from patients with well-characterized disorders. The most striking finding was that Alzheimer's disease produced a marked reduction in toxin binding in the globus pallidus but not in other regions of the brain.

## **Transmembrane Voltage and pH Gradients Facilitate the Translocation of Tetanus Toxin through Membranes devoid of Receptors.**

H. Bigalke, T. Binscheck, F. Bartels

Institute of Toxicology, Medical School of Hannover, 30623 Hannover, FRG

Tetanus toxin forms pores in lipid bilayers and neuronal plasma membranes. In order to examine to what extent pore formation is involved in the translocation of the toxin the actions of both light and heavy chains were investigated in chromaffin cells. This type of endocrine cell lacks binding sites for the toxin, but it contains synaptobrevin II which is the intracellular substrate of the metalloprotease, tetanus toxin. Therefore, a passage through the membrane was postulated for the toxin, when exocytosis was inhibited by the cleavage of synaptobrevin II. Under conditions identical with those under which tetanus toxin blocks release of hormones, the pore formation was recorded by measuring the conductance of inside-out patches from chromaffin cells using the patch-clamp technique. Both blockage of exocytosis and channel formation occurred only if the membranes were exposed to the toxin at a low pH. A voltage gradient promoted the effects: tetanus toxin induced pores when the membrane was clamped to a transmembraneous potential of 80 mV. Accordingly, blockage of exocytosis could be reduced by depolarising the membrane with 30 mM potassium. In a next step, membrane vesicles were produced on the tip of the recording pipette filled with a tetanus toxin solution of pH 5. In this configuration pore formation could be recorded when it occurred in the far-side membrane indicating that tetanus toxin had entered the vesicle from the pipette solution. Preloading the vesicles with polyclonal anti-tetanus toxin antibodies delayed pore formation. These results show that tetanus toxin can cross cell membranes without interacting with a receptor provided conditions correspond to those prevailing in endosomes. Using this route to enter chromaffin cells, the toxin cleaves its substrate, thereby inhibiting exocytosis. Passage through the membrane is probably associated with the formation of transient pores, while closure of the pores may result from the dissociation of the toxin molecule from the intracellular side of the membrane.

**Purification and Structure-Function Studies of a Hemagglutinin from Type A *Clostridium Botulinum***

F. -N. Fu, S. K. Sharma and B. R. Singh

Department of Chemistry, University of Massachusetts Dartmouth, N. Dartmouth, MA 02747

Botulinum neurotoxins (serotype A-E) are found in fluid culture or in food as large, stable complexes, and depending on the serotype, have molecular size up to 900 kDa. The complex is made of the neurotoxin and a group of non-toxic proteins. The non-toxic component varies from one protein (E, and F) to 7 associated proteins (A and B). It has been suggested that non-toxic component proteins provide the protection of neurotoxin from acidity and proteolytic attack while it passes through the digestive tract. The complex has also been reported with (A and B) or without (type E) the hemagglutination activity. Recent studies further suggested that these non-toxic components play significant genetic role in the toxin production.

A 33 kDa non-toxic complexing protein of type A botulinum neurotoxin has been isolated and purified in our laboratory. High content (22%) of hemagglutinin-33 is present in the whole complex, which may imply the importance of this protein. The hemagglutinin-33 has strong hemagglutinating activity, and shows high proteolytic resistance to trypsin digestion and high immunogenic reactivity to the anti-complex sera. A secondary structure analysis of the protein shows 74-77%  $\beta$ -sheet, 16%  $\alpha$ -helix and 7-10% random coil content. N-terminal a. a. sequence and the isoelectric point have also been determined. Matrix based laser desorption mass spectroscopic result reveals a high degree of oligomeric structure of hemagglutinin-33. (Supplied by NRL/ERDEC and USDA)

## THE STAPHYLOCOCCAL ENTEROTOXINS

Merlin S. Bergdoll

Food Research Institute, University of Wisconsin  
1925 Willow Drive, Madison, WI 53706 U.S.A.

Staphylococcal food poisoning is a common type that occurs world wide. The organism produces in the food a toxin that is heat stable and resistant to pepsin and trypsin. It was named enterotoxin because of its effect on the intestinal tract, producing vomiting and diarrhea. Although this food poisoning was reported as early as 1830, it was not associated with staphylococci until 1914 when Barber in the Philippines found milk made him ill only after staphylococci grew in the milk and produced a toxin. Dack et al.(1930) showed that staphylococci isolated from a Christmas cake that made several people ill did produce a toxin that made human volunteers ill with vomiting and diarrhea. It was called enterotoxin. Its chemical nature was reported by Bergdoll et al. in 1959; a newly developed ion exchange resin was used in the purification. Detection of the enterotoxin was by the reaction with specific antibodies in gels. The staphylococcal strain used for its production produced a second toxin that did not react with the specific antibodies to the purified enterotoxin. The second enterotoxin was called enterotoxin A (SEA) because it was the one associated with food poisoning. Seven enterotoxins have been purified, three of them SECs, with only slight differences in their immunological reactions. Cross-reactions occur between SEB and the SECs and between SEA and SEE. Two new enterotoxins have been identified, SEG and SEH. They are produced in small amounts and are not significant in food poisoning. One common structure in the enterotoxins is a cystine loop in the middle of the molecule. After the cystine loop is a common amino acid sequence. The amino acid sequences have been reported. The enterotoxins are called superantigens because of attachment to T-cells. They stimulate the production of cytokines.

## STRUCTURE-FUNCTION STUDY OF TOXIC SHOCK SYNDROME TOXIN-1 (TSST-1)

Peter Bonventre, Edmond Choi, Lorna Blanco, Holly Heeg and Constance Cullen:  
Dep't. of Molecular Genetics, Biochemistry and Microbiology, University of  
Cincinnati Medical Center, Cincinnati, Ohio, 45267, U.S.A. Staphylococcal toxic  
shock syndrome toxin (TSST-1) is a 22Kd protein linked with human TSS. In  
common with several enterotoxins, TSST-1 behaves as a superantigen and thus is a  
potent T cell mitogen. The activation of entire populations of T cells is believed to  
induce the shock like symptoms of TSS. Several years ago we began a structure-  
function study of TSST-1 utilizing a mutational analysis. Site directed mutants  
whereby histidine and tyrosine residues were replaced by alanine were tested for  
retention of mitogenicity, reactivity with TSST-1 specific monoclonal antibodies  
and toxicity as measured in several animal models of TSS. The early studies  
revealed that the region between amino acids 115 and 141 of TSST-1 constitutes  
a domain critical for biological activity. Several mutations within this region  
resulted in partial loss of mitogenic activity and/or loss of reactivity with specific  
antibodies. One mutant toxin proved to be unique. Modification of histidine  
residue 135 resulted in a modified toxin (H135A) which was completely devoid of  
mitogenic activity, retained reactivity with monoclonal antibody, and appeared to  
be of much diminished toxicity. Since H135A possessed characteristics of a  
potential vaccine candidate it was subjected to further analysis. In the rabbit model  
of TSS, viable staphylococci are inoculated into s.c. chambers; recombinants  
expressing unmodified TSST-1 caused lethal shock while the H135A mutant did  
not cause significant illness. The H135A mutant retained immunogenicity and was  
not significantly altered in its conformation. H135A elicited antibodies which were  
cross-reactive with native TSST-1 and which neutralized the mitogenic potential of  
the superantigen. Additionally, H135A antibodies blocked the production of TNF  
by murine spleen cells stimulated with wild-type TSST-1. The diminished toxicity  
of H135A was confirmed in two distinct murine models of superantigen induced  
lethal shock. Finally, in the rabbit infection model of TSS, H135A antibodies  
administered passively, protected rabbits challenged with *Staphylococcus aureus*  
producing wild-type recombinant TSST-1. Since H135A was found to bind  
normally to MHC class II positive cells we conclude that the loss of biological  
activity of the toxin mutant is due to a defect in its interactions with the T cell  
receptor.

INHIBITION OF STAPHYLOCOCCAL ENTEROTOXIN B (SEB)-STIMULATED  
MITOGENICITY IN HUMAN T-CELL CULTURES BY BLOCKING  
LIPOXYGENASE, BUT NOT CYCLOOXYGENASE PATHWAYS.

Marti Jett, Chris Welch, Sheila McMorrow, Thomas Boyle, David Hoover, Peter  
Gemski. Walter Reed Army Inst. Res. Washington, D.C.

The *S. aureus* enterotoxins are classified as superantigens based on their ability to stimulate large numbers of T-cells. In addition to enterotoxic activities, these toxins have been observed to induce lethal shock in man and non-human primates. SEB displays mitogenicity in the classic model, mouse spleen cell cultures, as well as in the more sensitive model utilizing human T-cell/monocyte cultures (Jett et al, 1995. Infect. Immun 62:3408-3415). Previous biochemical studies both in vitro and in vivo had indicated that arachidonate metabolism was markedly stimulated in the presence of SEB. Therefore, we examined the effectiveness of numerous inhibitors of arachidonate metabolism. The assay system used was human T-cell/monocyte cultures. The cell cultures were pre-incubated in the presence of the inhibitor for 1hr; SEB was added, incubation continued for 72 hr and <sup>3</sup>H-thymidine incorporation determined. The cyclooxygenase inhibitors, aspirin and indomethacin, were completely inactive up to 50  $\mu$ M; this correlated with biochemical studies in vitro in which SEB did not stimulate production of prostanoids. Inhibitors which blocked both cyclooxygenase and lipoxygenase activity (eicosatetriynoic acid, phenadone and dexamethasone) were only active at relatively high concentrations. General lipoxygenase inhibitors (nordiguaretic acid) and 5-lipoxygenase(LO) inhibitors (AA861, ketoconazole, etc) prevented SEB-induced mitogenesis at low concentrations (Previous studies showed that 5-LO products were rapidly produced in response to SEB.). In addition, inhibitors of protein kinase C (PKC) (sphingosine, H7, chelerythine) effectively inhibited SEB-stimulated proliferation, a confirmation of our previous studies which showed that SEB-induced formation of surface markers reflective of PKC activation. Inhibitors of tyrosine kinases (tyroprostin, genistein) were not inhibitory even at high concentrations, despite reports suggesting a role of these enzymes in SEB-induced mitogenesis. Our current and previous studies demonstrate that 5-LO metabolites were produced in response to SEB, and inhibition of the 5-LO pathway blocked this biological response, SEB-stimulated proliferation of human T-cells.



**Binding and Uptake of Anthrax Toxin Components and Fusion Proteins by Eukaryotic Cells.** S. H. Leppla, K. R. Klimpel, V. M. Gordon, N. Arora, and Y. Singh, Laboratory of Microbial Ecology, National Institute of Dental Research, NIH, Bethesda, MD 20892.

*Bacillus anthracis* produces three proteins which combine to form two toxic activities. Protective antigen (PA, 82 kDa) and edema factor (EF, 90 kDa) combine to form edema toxin (ET), while PA and lethal factor (LF, 90 kDa) combine to form lethal toxin (LT). PA binds to a unidentified receptor present on the surface of most types of cells. PA bound to receptor is cleaved at a single site by the cell-surface protease furin to produce a carboxyl-terminal 63-kDa fragment (PA63) that remains bound to the cell surface. Only after cleavage is PA able to bind either EF or LF to form either ET or LT. Mutagenesis and preliminary X-ray diffraction data has identified four domains in PA and assigned functions to each. The central translocation domain has homology to *Clostridium perfringens* iota toxin. A detailed analysis of the domains of LF identified the amino-terminal 254 amino acids as the region that binds to PA63. Thus, fusion proteins containing residues 1-254 of LF and the ADP-ribosylation domain of *Pseudomonas* exotoxin A (PE) are highly toxic to cultured cells, but only when PA is administered simultaneously. Similar fusions were made to the catalytic domains of diphtheria toxin, Shiga toxin, and tetanus toxin. Each of these fusion proteins is highly toxic to cultured cells, showing that the membrane translocation function in PA is very efficient and can accommodate a number of different polypeptides. Studies of the PA receptor and the role of furin in activation have been facilitated through selection of toxin-resistant CHO cell mutants. A number of mutants deficient in receptor or furin have been obtained which are being used to identify target cell processes involved in intoxication.

## SHIGA-LIKE TOXINS OF ENTEROHEMORRHAGIC *E. coli*

Alison D. O'Brien, Dept. of Micro and Immuno., USUHS,  
Bethesda, MD., U.S.A., 20814-4799

Shiga-like toxins (SLTs), or Verotoxins, are essential virulence determinants of enterohemorrhagic *Escherichia coli* (EHEC), organisms responsible for food-borne outbreaks of hemorrhagic colitis and the hemolytic uremic syndrome (HUS). The SLTs are members of a family of toxins that share a number of traits. Each SLT is comprised of one enzymatically active A subunit and a pentamer of B or cell-binding subunits. The A and B subunit toxin genes are contained in an operon located on the bacterial chromosome or a lysogenic phage. The SLTs are cytotoxins that inhibit protein synthesis in eucaryotic cells by cleaving a single purine from 28 S rRNA. Members of the SLT family bind preferentially to globotriaosylceramide (Gb<sub>3</sub>) or Gb<sub>4</sub> on eucaryotic cells. There are two major immunologically non-cross reactive groups of SLTs within the family, SLT-I and SLT-II. The amino acid composition of SLT-I is nearly identical to that of Shiga toxin (STX) from *Shigella dysenteriae* type 1. No significant antigenic or receptor-binding differences within the STX/SLT-I group have been reported, in contrast to the SLT-II group. To compare the biological activities of the SLTs *in vivo*, we used a model in which SLT-producing *E. coli* were fed to streptomycin-treated mice. Animals infected with certain SLT-producing *E. coli* developed toxin-mediated renal tubular damage and died. In this seminar, I will review our findings that SLT-II-producing EHEC are more virulent than those that make SLT-I and that enhanced pathogenicity among the SLT-II-producing variant strains correlates with increased toxin activity in the presence of small intestinal mucus.

## CHOLERA ENTEROTOXIN (CT) AND CT-LIKE ENTEROTOXINS

Richard A. Finkelstein

School of Medicine, University of Missouri,  
Columbia, MO 65212

Cholera enterotoxin (CT), responsible for the life-threatening diarrhea of cholera, was discovered by S.N. De and N.K. Dutta in India in 1959 and purified to homogeneity in our laboratory in 1969. A number of other bacteria, most notably enterotoxigenic *Escherichia coli*, have since been found to cause diarrhea by means of CT-like heat labile enterotoxins or LTs. These are bipartite 84 kDa proteins consisting of a 28 kDa A (active) subunit and 5 identical 11.5 kDa B (binding) subunits associated non-covalently. Although the B subunits of the family are the immunologically dominant portion of the holotoxins and are related to each other, they are not identical and can be differentiated readily by monoclonal antibodies. Cholera vaccines have been in use since 1885, but a suitable vaccine which is economical, effective, and provides long-lasting immunity without side effects remains to be developed and deployed. Results of field studies of toxoid vaccines and of the B subunit protein added to killed whole cells and administered orally have been disappointing perhaps, in part, due to failure to recognize that there are two major epitopes of CT, CT-1 and CT-2, and that cholera vibrios have additional mechanisms for causing (milder) diarrhea. We have recently genetically engineered CT-1 and CT-2 with amino acid substitutions in the A subunit which make them biologically inactive. Living attenuated strains carrying these analog toxins or conjugates consisting of *Vibrio cholerae* polysaccharide (from LPS) linked to the toxin analogs may be useful as vaccines. One chimeric construct contains both CT-1 and CT-2 B subunit proteins.

## **Structural Aspects of Ricin Bearing on Drug Design and Membrane Translocation.**

Jon D. Robertus, Jörg Steighardt, Maria Svinth, Phillip Day, Arthur Monzingo, and Stephen Ernst. Department of Chemistry and Biochemistry, University of Texas, Austin TX 78712

The X-ray structure of heterodimeric ricin and the cloned A chain (RTA) have been determined previously to high resolution. A catalytic mechanism has been proposed based on the structure and on analysis of site-directed mutants. Work is continuing on a number of related topics including: 1) the X-ray structure of site-directed mutants bearing on the proposed mechanism of this medically important family of proteins, 2) the mode of membrane translocation, and 3) the design of inhibitors based on the X-ray structure.

1) The crystal structure of several mutants, including that converting the key active site Arg 180 to His have been solved. The mutant protein is fully active when fresh but has been selected against because it is thermodynamically metastable. 2) To date the mode by which the toxin crosses the membrane to reach target ribosomes in the cytoplasm has not been determined. Site-directed mutagenesis is being used to investigate translocation and has revealed a role for a very hydrophobic sequence near the C terminus. In particular the conversion of Ile 251 to Arg reduces membrane translocation over 100 fold. 3) There is considerable interest in identifying or designing specific inhibitors of ricin A chain based on the active site structure. A number of data base searches using programs DOCK and UNITY have been carried out to identify inhibitor lead compounds. These studies will be described along with crystallographic analysis of several compounds.

THE CYTOTOXIC RIBONUCLEASE  $\alpha$ -SARCIN: THREE-DIMENSIONAL STRUCTURE; RIBOSOMAL RNA IDENTITY ELEMENTS FOR SPECIFIC RECOGNITION; AND AN ENGINEERED CHANGE IN THE RNA SUBSTRATE THAT AFFECTS ENZYME SPECIFICITY. **Ira G. Wool**, Department of Biochemistry and Molecular Biology, The University of Chicago, 920 East 58th Street, Chicago, Illinois 60637 USA.  $\alpha$ -Sarcin is a cytotoxic ribonuclease that inactivates ribosomes by hydrolyzing the phosphodiester bond on the 3' side of G-4325 in a universal sequence in 28S rRNA. Ribosomal RNA has approximately 7,000 phosphodiester bonds but the enzyme cleaves only this one. We have defined the identity elements for recognition of this domain using an oligoribonucleotide (35-mer) that reproduces the sequence and the structure of the domain (a helix, a bulged nucleotide, and a loop) that contains the site of modification. The wild type and a large number of variants were transcribed *in vitro* from synthetic DNA templates with T7 RNA polymerase. The critical structural element for  $\alpha$ -sarcin recognition is G-4319 in the universal sequence which forms a distinctive structure. The conformation of the  $\alpha$ -sarcin stem-loop has been determined by NMR spectroscopy. In addition, our colleagues Xiaojing Yang and Keith Moffat have obtained the three-dimensional structure of restrictocin (which shares 85% amino acid sequence identity with  $\alpha$ -sarcin) at 1.7 Å resolution by X-ray diffraction of crystals. Docking experiments with restrictocin and the  $\alpha$ -sarcin rRNA stem-loop have been carried out. Finally, the  $\alpha$ -sarcin domain RNA has been altered in a way that confers on the enzyme a new, specific cleavage site. This leads to a prediction of how the toxin orients itself on the substrate and selects the site of covalent modification.

## Nucleoside N-Ribohydrolase Inhibitor Design Applied to Ricin A-Chain

Todd Link, Xiangyang Chen, Lin-Hao Niu and Vern L. Schramm  
Department of Biochemistry, Albert Einstein College of Medicine  
Bronx, NY 10461

Ricin A-chain catalyzes the depurination (N-ribohydrolysis) of adenosine at position 4324 of mammalian 28s rRNA, to destroy the binding site of eukaryotic elongation factor 2. One ricin molecule is sufficient to depurinate all ribosomes in a single cell, making ricin one of the most potent biological toxins. Enzymatic depurination reactions for AMP and inosine are catalyzed by AMP nucleosidase and nucleoside hydrolases and provide chemical models for the N-riboside bond hydrolysis of ricin. The transition states for both enzymes have been established by kinetic isotope effect measurements. At the transition state, the enzymes stabilize  $S_N1$ -like transition states with well-established oxycarbonium character in the ribosyl, protonated purine leaving groups and enzyme-activated incipient water nucleophiles. Inhibitors with geometric and electronic similarity to the transition state are powerful inhibitors of these N-ribohydrolases. Since the depurination catalyzed by ricin is chemically equivalent to these reactions, inhibitors were designed to incorporate oxycarbonium or protonated leaving group analogues into RNA stem-loop structures. RNA stem loops containing formycin 5'-phosphate and phenylimino 5'-phosphoribitol were synthesized using phosphoramidite chemistries. Monomeric analogues and analogues in stem-loop RNA show weak inhibition of ricin A-chain action on *in vitro* translation assays. These results, together with the kinetic properties of ricin A-chain, suggest that the enzyme is not effective in forming transition state complexes in binary mixtures or in achieving the transition state in reaction mixtures.

Supported by research contract DAMD 17-93-C-3051 from the United States Army Medical Research and Materiel Command.

## Ribosome Inactivating Enzymes in Castor Bean and Man

Richard J. Youle

NINDS, NIH, Bethesda, MD 20892

Ricin, an extremely toxic protein found in castor beans, is related to toxic proteins found in a wide variety of plants. These proteins cleave ribosomal RNA to effect cytotoxicity. Subcellularly ricin is found compartmentalized within the protein bodies in the cells of the seed and segregated from the plants own ribosomes. These organelles are derived from the vacuole which has features similar to the lysosomes found in animal cells. Within the protein bodies a large crystalline protein structure is found made up of nontoxic storage protein. Ricin and a homologous tetrameric lectin are found soluble, surrounding the crystalloid protein within the protein body membrane. In humans two toxic proteins are found within eosinophils. Interestingly, these proteins also exist within membrane bound protein-rich vesicles, surrounding other proteins that form crystalloid inclusion bodies. Named eosinophil derived neurotoxin and eosinophil cationic protein, these two proteins are 50% identical in sequence and selectively and potently kill neurons when injected into the central nervous system. Their sequence reveals similarity to the pancreatic family of ribonucleases and they indeed express ribonuclease activity. Ribonuclease activity appears necessary for cytotoxicity and we propose that these proteins enter cells and degrade RNA to effect cell death. These proteins may function in host defense.

## PLANT TOXINS

Gerhard G. Habermehl

Department of Chemistry, School of Veterinary Medicine  
D-30173 Hannover, Germany

Although plant toxins have been known since ancient times, they did not find the same interest as toxins from animals as a hazard. Quite to the contrary: Many of those plants have found their way to food, and herbal medicine, despite of the fact that they may act as hepatotoxins, neurotoxins or cardiotoxins. Plants of the genus Senecio play their role as the cause of seneciosis in animals, and via the food chain in man. The extend of such symptoms depends highly on the different species of animals, and their ability to metabolize these substances, or even to detoxify them. Other plants are recommended as food because of their content in proteins, fat, or carbohydrates, like the Brazilian plant Prosopis juliflora. This plant causes severe damages to the central nervous system in cattle, similar to bovine spongiforme encephalitis, due to its content of alkaloids. Care should be taken, therefore, in recommending this plant as human food reserve. It is, moreover, noteworthy to keep in mind that also fungi may be the origin of plant toxins; a good example being the Brazilian plant Baccharis coridifolia. Its toxins, trichothecenes, are prepared by the fungus imperfectus Myrothecium verrucaria living close to the roots of this plant, as ektomycorrhiza.



## TOXIGENIC FUNGI IN THE AIR WE BREATHE

Bruce B. Jarvis, Department of Chemistry and Biochemistry University of Maryland, College Park, MD 20742

Mycotoxins are small molecule secondary metabolites produced by fungi which pose a risk to the health of humans and animals. Various toxigenic fungi (e.g. *Aspergillus*, *Fusarium*, *Penicillium*, etc.) are well known to contaminate food and feed and thus pose a hazard through ingestion of contaminated farm produce. What is less well appreciated is that water-damaged buildings can also be a source of toxigenic fungi which threaten the health of the occupants. Our current state of knowledge of the risks posed by airborne mycotoxins will be reviewed and current work will be presented on the hazards associated with *Stachybotrys atra*, a particularly troublesome fungus sometimes found growing in abundance in water-damaged buildings.

STRUCTURE-ACTIVITY RELATIONSHIPS FOR PHYTOTOXICITY AND  
MAMMALIAN CYTOTOXICITY OF THE SPHINGOSINE ANALOG  
MYCOTOXINS FUMONISIN B<sub>1</sub> AND AAL-TOXIN

<sup>1</sup>W.T. Shier, <sup>1</sup>F.A. Badria and <sup>2</sup>H.K. Abbas

<sup>1</sup>University of Minnesota, Minneapolis, MN 55455, U.S.A.; <sup>2</sup>Southern Weed  
Science Laboratory, USDA/ARS, Stoneville, MS 38776, U.S.A.

Fumonisin B<sub>1</sub> (FB<sub>1</sub>) is a mycotoxin produced by the ubiquitous corn contaminant *Fusarium moniliforme*, and by other fungi including *F. proliferatum*, *F. nygamai* and *Alternaria alternata*. AAL-toxin is a mycotoxin produced by *A. alternata*, which causes stem canker on susceptible tomatoes (*asc/asc*) and some other plants. Both toxins are structural analogs of sphingosine, and are thought to act either by inhibiting sphingolipid synthesis or altering the intracellular regulatory activities of sphingosine. The two toxins cause a similar spectrum of toxic responses in weeds and other plants, and in cultured mammalian cells. Concerns about the mammalian toxicity of FB<sub>1</sub>, which is known to cause leukoencephalomalacia in horses, pulmonary edema in swine and possibly cancer in rats, arose during exploration of the potential of these toxins as bioherbicides. Therefore, a series of structural analogs have been evaluated for retention of phytotoxicity and reduction of mammalian cytotoxicity. Structural analogs have been obtained from the following two sources: (i) chemical modifications on the structures of FB<sub>1</sub> and AAL toxin; and (ii) a series of analogs synthesized from oleic acid, which were provided by Dr. G.A. Kraus, Iowa State University, Ames, IA. They were examined for phytotoxicity by measuring chlorophyll loss and cellular leakage from excised leaf discs and by duckweed mortality (*Lemna pausicostata*) in xenic liquid culture, and for mammalian cytotoxicity with a series of cultured mammalian cell lines, some of which exhibit unusual susceptibility to FB<sub>1</sub> and AAL toxin. A free amino group and a long, hydroxylated alkyl chain was needed for activity in all systems, but beyond that a very wide range of structural variations retained substantial biological activity in all the test systems. Side chains were not required for activity. Ester derivatives retained greater activity in plants than in mammalian systems. Retention of mammalian toxicity in a wide range of structural analogs presents challenges for the development of this structural class of mycotoxins as bioherbicides, and raises concern that thermal degradation products generated by cooking foods contaminated with FB<sub>1</sub> may retain biological activity. (Supported in part by NRICGP/USDA)

## CYANOBACTERIAL BLOOMS AND HEALTH HAZARDS - AN OVERVIEW

Wayne W. Carmichael

Department of Biological Sciences, Wright State University, Dayton, OH 45435,  
U.S.A.

Cyanobacteria, or blue-green algae, are best known to water authorities, water utility managers and the general public for their adverse impacts on water quality, including the production of tastes or odors and interference with certain water treatment processes. To minimize these problems in the production of potable water, many water suppliers have developed strategies for managing or controlling the growth of cyanobacteria in water supply sources. It is also known that under conditions of extreme growth or bloom development, some species of cyanobacteria are capable of producing toxins at levels that may render a water unsafe for consumption by people or animals. Cases of livestock and wildlife poisonings due to "toxic blooms" are reported in the scientific literature as far back as 1878. In most of these cases, adverse health effects were only observable following accidental ingestion of water containing heavy growths of toxic organisms. As a general statement, acute lethal injury to humans from consuming cyanobacterial toxins in drinking water should not occur. This is because in the management of most domestic water supplies cyanobacteria are controlled to minimize operational and aesthetic problems, and normal water treatment processes should dilute toxic cells and released toxins to non-lethal levels. Recent evidence, however, suggests that part per billion levels of microcystins and nodularins may be associated with non-lethal acute or chronic health effects including tumor promotion and should be monitored and controlled in domestic water supplies. As a result, a few nations, including Canada, Australia and Great Britain are currently moving toward the development of health guidance levels for microcystins in drinking water. Microcystins have also been detected in aquacultured salmon, striped bass and shrimp. With salmon and bass they produce an acute lethal liver disease while in shrimp they can bioaccumulate to levels that may pose a threat to consumers.

RECENT ADVANCES IN COELENTERATE VENOM RESEARCH 1991-95: CLINICAL, CHEMICAL AND IMMUNOLOGICAL ASPECTS. Joseph W. Burnett, M.D., David Bloom, M.A., Shinichi Imafuku, M.D., C. Lisa Kauffman, M.D. Laure Aurelian, Ph.D., Samuel Morris, Ph.D., Baltimore & Columbia, MD, USA

Clinical, chemical and immunological advances in coelenterate venom research have been made in recent years. Rapid fatal hepatocellular damage, self resolving incidents of mononeuritis multiplex and superficial local cutaneous thrombophlebitis have followed envenomation. Seabather's eruption has been found to be due to Linuche unguilata planula larvae. Capillary electrophoresis, a technique separating venoms into fractions, has advanced our understanding of coelenterate venom chemistry. The major protein toxins of Chrysaora quinquecirrha are immunologically active and binds antibody within seconds as demonstrated by alteration in capillary electrophoresis. Additional insight into the pathogenesis of stings has resulted from immunological studies. Syndromes ordinarily associated with immunological mechanisms (urticaria, persistent nodules) appear in patients with unusual T-cell responses. T-cells from envenomated patients proliferate when exposed to jellyfish venom proteins. This proliferation is mediated by both Th<sub>1</sub> and Th<sub>2</sub> cells because it can be inhibited by sera containing antibodies to interleukin 2. In mouse experiments topical ultraviolet light treatment, administered either before or after venom exposure, reduced the in vitro immunological activity of internal splenocytes and regional lymph node cells. Ultraviolet light administered in vivo or in vitro can also decrease the proliferative response of human circulating lymphocytes to jellyfish antigens. This decrease in cellular proliferation extends broadly to include responses against exposure to pathogens, mitogens and endogenous proteins. Both ultraviolet light and the coelenterate venom itself detrimentally alter human immunity by various means including directly affecting circulating T-cells. Since venoms act by immunological as well as toxic pathways, any abrogation of normal immunity could enhance their action on man.

# IDENTIFICATION OF ESSENTIAL RESIDUES IN THE POTASSIUM CHANNEL INHIBITOR ShK TOXIN: ANALYSIS OF MONOSUBSTITUTED ANALOGS

William R. Kem, Michael W. Pennington<sup>1</sup>, Vladimir M. Mahnir,

Michael E. Byrnes<sup>1</sup>, Inna Zaydenberg<sup>1</sup>, Ilya Khaytin<sup>1</sup>,

Doug S. Krafte<sup>2</sup> and Roger Hill<sup>2</sup>, and Jose de Chastonay<sup>1</sup>

Department of Pharmacology and Therapeutics, University of Florida,  
Gainesville, FL 32610, <sup>1</sup>Bachem Bioscience Inc. 3700 Horizon Dr., King of  
Prussia, PA 19406, <sup>2</sup>Sterling-Winthrop, Box 3000, Collegeville, PA 19426

ShK toxin (RSC<sup>3</sup>IDTIPKSRCS<sup>12</sup>TAFQC<sup>17</sup>KHSMKYRLSFC<sup>28</sup>RKTC<sup>32</sup>-GTC<sup>35</sup>), isolated from the sea anemone *Stichodactyla helianthus*, is a potent blocker of the lymphocyte Kv1.3 channel and brain K<sup>+</sup> channels. Monosubstituted analogs of the native toxin sequence were prepared in order to identify those residues which are important in binding to the K<sup>+</sup> channel receptor. These included Arg<sup>1</sup> to Ser; Asp<sup>5</sup> to Asn; Lys<sup>9</sup> to Gln; Arg<sup>11</sup> to Gln; Phe<sup>15</sup> to Ala or Trp; Lys<sup>22</sup> to Ala or Arg; Tyr<sup>23</sup> to Ser or Phe, and Arg<sup>24</sup> to Ala. Biological activity was determined on Jurkat T lymphocyte Kv1.3 channels (patch-clamp recording and iodinated charybdotoxin binding) and rat brain K<sup>+</sup> channels (displacement of iodinated dendrotoxin). The Ser<sup>1</sup>, Ala<sup>15</sup>, Trp<sup>15</sup>, Phe<sup>23</sup>, and Ala<sup>24</sup> analogs each had IC<sub>50</sub> values nearly equivalent to the native toxin. The Gln<sup>9</sup> and Gln<sup>11</sup> analogs had slightly lower IC<sub>50</sub> values (reduced by 5- to 10-fold) than native ShK for lymphocyte but not rat brain K<sup>+</sup> channels. The Ala<sup>22</sup> and Ser<sup>23</sup> analogs had IC<sub>50</sub> values that were reduced by >500-fold on rat brain channels, and the Arg<sup>22</sup> analog showed a 100-fold reduction in IC<sub>50</sub> for displacement of radiolabelled dendrotoxin binding to rat brain synaptosomes. We have thus identified two regions of ShK sequence that are likely to be part of the toxin pharmacophore for interaction with these K channels.

ShK blocks Kv 1.3 channel. T-lymphocytes are used for the channel in culture.

317.  $\alpha$ -helix

187.  $\beta$ -sheet

177.  $\beta$ -turn

remainder of the

Bob William

ShK competitively inhibits charybdotoxin by Scatchard Plot

ShK is a noncompetitive binding to  $\alpha$ -DTX.

Change the amino terminal R<sup>92</sup> & K<sup>9</sup> & { Change IC<sub>50</sub> to lower ability to compete

**CARDIOVASCULAR EFFECTS OF PHYSALIA VENOM.** David Hessinger.  
No Abstract.

## BIOLOGICAL AND BIOCHEMICAL STUDIES ON THE VENOM OF THE POISONOUS FISH *THALASSOPHRYNE NATTERERI* (NIQUIM).

M. L. Ferreira, K. C. Barbaro, D. F. Cardoso and I. Mota

Lab. of Immunopathology - Butantan Institute - 05503-900 - S. Paulo - SP  
- BRAZIL.

Accidents caused by the poisonous fish "niquim" (genus *Thalassophryne*) are common in the Brazilian shores particularly in the northeast. The biological and biochemical properties of its venom are for the most part unknown. Victims stung by these fishes present erythema, oedema and necrosis in the affected member. The patient has fever and complains of intense pain. In some cases, secondary infection may require amputation of the affected member. We present here preliminary data on the biological and biochemical properties of the venom. The results of SDS-PAGE analysis of the crude venom showed at least 17 components, with the major band being located around 19,000 mol. wt. Almost all antigens stained were also revealed by Western blotting with antibodies to *T. nattereri* venom. The toxic activities of *T. nattereri* venom was studied using mice as an experimental model. Injected i.d. the venom induced erythema and oedema followed by hemorrhage and necrosis. *T. nattereri* venom showed low level of proteolytic activity and no detectable phospholipasic activity (even using large amounts of venom). The dose of venom to kill 50% of mice within 48 hr was 4,54 mg/kg body weight. The neutralizing ability of the anti-*T. nattereri* serum was estimated after *in vitro* incubation of venom with antiserum. All toxic activities were 100% neutralized after incubation with antiserum except oedema. These results suggest that serum therapy may be used as treatment to counteract the effect of the venom.  
Support: I. Butantan, FAPESP (94/1517-4).

## MASS SPECTROMETRIC INVESTIGATIONS ON CONUS PEPTIDES

*L. Krishnamurthy & M. Prabhakaran*

*U.S. Army Edgewood RD&E Center, Aberdeen Proving Ground, MD  
21010-5423, USA*

Several neurotoxic (conotoxins) and biologically active (conopressins & conantokins) conus peptide standards were introduced, either directly or after a LC separation, into the electrospray ionization source. The molecular masses could be measured very accurately using only femtomolar amounts. The disulfide bridges were reduced and the resulting mercapto groups were derivatized with iodoacetic acid. The carboxymethyl derivatives were ionized, the selected multiply charged precursor ions were subjected to collisionally induced dissociation using argon and the resultant daughter ions were detected. The amino acid sequences could be determined from the recorded daughter spectra. Derivatization and the mass spectrometric procedures were optimized. Extreme care was required to minimize the sample loss due to adsorption phenomenon displayed by these intensely hydrophilic molecules. The developed method was applied to investigate an unknown conus snail venom from Bay of Bengal region. The components of the *Conus betulinus* venom mixture was separated and purified over a size exclusion followed by reverse phase column chromatography. Over 50 different fractions were collected and each fraction contained multiple components. The amino acid and MS analysis of several fractions indicated them to be small cysteine rich peptides. The measured molecular masses ranged between 1 to 3.5 kDaltons.



## COMPARATIVE TOXINOLOGY OF VENEZUELAN SPONGES FROM THE GENUS *Haliclona*

C. Sevcik, M. Ramos and G.D'Suze

Instituto Venezolano de Investigaciones Científicas, Centro de Biofísica y  
Bioquímica, Laboratory of Cellular Neuropharmacology, Apartado 21827  
Caracas 1020-A Venezuela.

A toxin (HvTX) able to selectively block potassium channels in the resting membrane of nerve and muscle has been isolated from *Haliclona viridis* (Sevcik *et al.*, 1986, 1988, 1994; Jaffé *et al.*, 1993). We have now studied the toxins present in *H. crassiloba*, *H. doria*, *H. permollis* and *H. variabilis* collected from mangrove forests, the same habitat of our initial samples of *H. viridis*. It was possible to isolate depolarizing compounds from all the species of *Haliclona* tested, but most of them were readily reversible, of low potency and had an elution pattern in BioGel P2® (BioRad Labs., Richmond, CA) different than HvTX. Only one fraction isolated from *H. variabilis* was similar to HvTX in potency and in the tight binding to muscle that made it difficult to wash. It was also possible to isolate toxin  $\beta$  produced by the dinoflagellate *Ceratium furca* (Mijares *et al.*, 1985), from the *Haliclona* species growing in mangrove forests, as it was done previously from the sponge *Tedania ignis* in the same habitat (Sevcik and Barboza, 1982). Toxins isolated from sponges are often accumulated from planktonic microorganisms or from bacteria living within the sponges. Since all the sponge samples in this study were collected from mangrove roots, our results show that HvTX is produced by *H. viridis* and perhaps also by *H. variabilis*. This conclusion agrees with experiments in which HvTX was isolated from *H. viridis* collected from coral reefs in several Caribbean islands (Sevcik *et al.*, 1988). [Partly funded by CONICIT (Venezuela) grant S1-2086].

PATHOPHYSIOLOGY OF A NOVEL PROTEIN UpI FROM THE  
SEA ANEMONE *URTICINA PISCIVORA*

E. I. Cline, Michael W. Wolowyk, D. Biggs and L. I. Wiebe  
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta  
Edmonton AB CANADA T6G 2N8

UpI is a lethal protein that has been isolated and characterized using a combination of chromatographic and biochemical methods, from the sea anemone *Urticina piscivora* collected from the west coast of Canada. It is a basic protein,  $pI > 9.3$  with a molecular mass ( $\sim 28$  kDa) and a partial N-terminal sequence, D<sup>1</sup>ENEN<sup>5</sup>LYGPN<sup>10</sup>ENKAK<sup>15</sup>AKDLT<sup>20</sup>AGASY<sup>25</sup>LTDEA<sup>30</sup>GCTKL<sup>35</sup>QAGCT<sup>40</sup>MYQAY<sup>45</sup>N. Pharmacological evaluation have revealed UpI to be a very potent cardiac stimulatory protein ( $ED_{50}$   $8.1 \times 10^{-9}$  M) comparable to isoproterenol on rat left atria, a potent hemolysin on erythrocytes of five mammals species and is cytotoxic to three cell lines, KB, L1210 and HEL 299 cells *in vitro*. However i.v. injection of UpI to anaesthetized rats produced severe hypotension at concentrations as low as  $0.5 \mu\text{g/kg}$  and causing death within 10-15 min. of administration. Pathophysiological studies revealed extensive hemorrhage and necrosis of the lungs, abdominal region and extremities of the skin, liver and kidney but no histological alterations to the heart. UpI is a new class of sea anemone cardiac stimulant protein which also belongs to the class of cytolytins ( $\sim 30$  kDa) for which no sequence information is yet available.

Fluorescent Labeled  $\alpha$ -Conotoxin GI: Binding Interactions  
with the Nicotinic Acetylcholine Receptor  
and Monoclonal Antibodies

J.D.Ashcom and B.G.Stiles

Fort Detrick USAMRIID Toxinology Division  
Frederick, MD, USA 21702

The  $\alpha$ -conotoxins, obtained in nature from the venom of predatory marine snails, are a structurally related family of low molecular weight polypeptides which bind to the nicotinic acetylcholine receptor and cause rapid muscular paralysis and death in animals. These peptides contain 13-15 amino acids with two intramolecular disulfide bonds. A fluorescent labeled derivative of  $\alpha$ -conotoxin GI has been prepared which binds with the receptor and also with protective monoclonal antibodies. Unlabeled ligands block the specific binding of the fluorescent peptide. This material has been used in a quantitative binding assay system which features solution phase interaction with conotoxin binding proteins followed by the rapid separation of unbound ligand. The fluorescent signal is linearly dependent on ligand concentration over a wide range and calibration curves permit the determination of molar binding ratios. These techniques could be used to measure low molecular weight ligand binding interactions in other systems.

# Novel Primary Structure of Sticholysin and its Interaction with Membranes.

C. Alvarez, M. Tejuca, \*V. Morera, \*V. Besada, F. Pazos, M.E. Lanio, \*G. Padrón Universidad de La Habana y \*Centro de Ingeniería Genética y Biotecnología, Havana, Cuba.

Sticholysin-I (St-I) is a basic 18 kDa polypeptide purified from the anemone *Stichodactyla helianthus* that exhibits hemolytic activity ( $HC_{50}$  -25-30 ng/mL). Amino acid sequencing showed the same amino acids up to cycle 29 that C-III (Blumenthal and Kem, 1983). However, from cycle 30 to 51 the aminoacids detected did not match with those of C-III but were identical again after cycle 51 to 67. Sequence alignments revealed more than 65 % homology with Eq-II from *Actinia equina* that also contains the 22 amino acid insert. St-I forms a hydrophilic pore of 1 nm internal radius. Induced hemoglobin or calcein release from cells or LUV-liposomes exhibited a Hill coefficient  $> 1$  indicating cooperativity among St-I monomers. Nevertheless, preincubation of St-I in a saline medium provoked a decrease in cooperativity. Evaluation of conformational changes could not detect any difference between the polypeptide preincubated or not in saline suggesting that potentiation is not mediated by preaggregation in solution. The enhancing rol of the medium ionic strength on the hemolytic capacity of St-I might be related with a transition to a more relaxed conformation that could interact more efficiently with membranes demanding less confluence of other St-I monomers to cause lysis.

Purification, Characterization and Immobilization of Proteinase Inhibitors from *Stichodactyla helianthus*

J. Delfin<sup>1</sup>, J. Díaz<sup>1</sup>, W. Antuch<sup>2</sup>, R. Rodríguez<sup>2</sup>, Y. González<sup>1</sup>, V. Morera<sup>2</sup>, I. Martínez<sup>1</sup>, N. Larionova<sup>3</sup>, G. Padrón<sup>2</sup> and M. Chávez<sup>1</sup>

<sup>1</sup>Facultad de Biología Universidad de La Habana. <sup>2</sup>Centro de Ingeniería Genética y Biotecnología La Habana Cuba. <sup>3</sup>Faculty of Chemistry, Moscow State University, Rusia.

The existence of proteinase inhibitors from *Stoichactis* sp. (*Stichodactyla* sp.) has been reported (Mebs and Gebauer, 1980; Chávez *et al.*, 1985). Proteinase inhibitors were isolated from the sea anemone *Stichodactyla helianthus*. The aqueous extract was previously treated with trichloroacetic acid followed by affinity chromatography on Trypsin-Sepharose and either Gel Filtration on Sephadex G-50 or Ion Exchange Chromatography on CM-Cellulose. The isoelectric point of the major inhibitor (ShPI) is 8.4 and the average molecular weight obtained by Fast Atom Bombardment (FAB-MS) is 6110 Da. The amino acid sequence was determined by FAB-MS combined with manual Edman degradation, endo and exo peptidases treatments and automatic sequencing. The sequence of ShPI (55 amino acids) was compared with those reported in the Protein Data Bank for several proteinase inhibitors; significant similarity to inhibitors belonging to the Kunitz family was observed. ShPI exhibits a broad specificity for serine, cysteine and aspartic proteinases. The dissociation constant of the complexes formed with different enzymes were determined. The affinity purified fraction was immobilized in different supports and similar results were obtained with Sepharose and Cellulose.

INSECT VENOM ALLERGENS    T.P. King, Rockefeller University, New York, NY 10021

Insect sting allergies to fire ants, bees and vespids are common in U.S. The vespids include hornets, yellowjackets and wasps. Susceptible people can become sensitized following a single sting of  $<10\mu\text{g}$  of venom proteins and they develop venom-specific IgEs. The major allergens from these insects have been sequenced and/or cloned by various investigators. Our studies are mainly with the vespid allergens. Insect allergens are proteins of 120-340 amino acid residues. Some insect allergens are structurally and biochemically similar but some are not. For example, both bee and vespid venoms contain hyaluronidases with a high degree of sequence similarity but their phospholipases differ markedly in their sequences and their enzymatic specificities. Another venom protein of unknown biological function, designated as antigen 5, is present in vespids and fireants but not in bees. One common feature of the venom allergens is their varying extents of sequence similarity with other proteins in our environment. For example, vespid antigen 5s and hyaluronidases have sequence similarities with mammalian testes proteins, and vespid phospholipases are similar with mammalian lipases. Some regions of sequence similarity are of sufficient length that they may function as cross reacting T and B cell epitopes. Recombinant fragments of venom allergens can be expressed in good yield in bacteria, and they retain the T cell epitopes of allergens. As they lack the native conformation of natural allergens, they do not bind, or bind poorly, allergen-specific antibodies which recognize mainly B cell epitopes of the discontinuous type. Thus the recombinant fragments are of low allergenicity and they may be useful reagents for immunotherapy. Others have reported that subcutaneous treatment of mice with the dominant T cell epitopes of allergens induce suppression of allergen-specific antibody response.

## **ANALYSIS OF THE STRUCTURE OF VENOMOUS PROTEINS FROM PARASITIC INSECTS**

Davy Jones, Graduate Center for Toxicology, University of Kentucky,  
Lexington, KY 40506

Insects parasitic on other insects, such as parasitic wasps, inject a number of regulatory agents into target tissues. The parasitic wasps in the subfamily Cheloniinae inject a number of interesting venom proteins into host caterpillars. These include a chitinase that is stored in active form in the chitin-lined venom reservoir, and a 33 kDa protein with an internal repeating structure. Analysis of the structure and activity of these proteins will be reported.

Insects that parasitize human beings also inject regulatory materials in their saliva into their hosts. While the components of the saliva of many blood-sucking arthropods has been analyzed in detail, virtually nothing is known about the molecular structure of components of the saliva of human body lice. We have initiated analysis of these molecules, and the results will be reported.

Supported, in part, by NIH GM 33995 and funds from the University of Kentucky program in Research and Graduate Studies.

## ENDOCRINOLOGICALLY ACTIVE VENOM PROTEINS OF ECTOPARASITIC WASPS

Thomas A. Coudron

USDA, ARS, BCIRL, 1503 S. Providence, Rsrch Pk, Columbia, MO 65203

Parasitism by hymenopteran species is a complex phenomenon; affecting the behavior, physiology and development of its host insect. The adult female parasitoid has the capability of regulating the host for the advantage of her offspring in order to provide a suitable source of nutrition and dwelling. Ectoparasitoids often rely on a venomous material produced by specialized tissues to regulate their hosts. Venomous substances of ectoparasitoids often affect the central nervous system or the endocrine system of the host. One of the better studied non-paralyzing venoms from an ectoparasitoid is produced by *Euplectrus* species in the family Eulophidae. The physiological effect of their venom is unique and causes an arrestment of the larval-larval molting process in the host. The substance arrests apolysis and ecdysis of the larval cuticle of the host. The occurrence of molt arrest in isolated tissues indicates that the venom may have a direct action on epidermal tissues, by gene regulation, thereby causing a cessation of cell division and an arrestment of ecdysis. The venom also causes processing of the host ecdysteroid milieu. However, arrestment is not reversible with treatments of juvenile hormone, methoprene, or 20-hydroxyecdysone. This indicates that the venom of *Euplectrus* spp. causes a permanent antiecdysteroid effect on the host. To date, only Eulophidae species and baculoviruses are known to arrest larval ecdysis in their hosts without causing paralysis. The pathogenic baculoviruses accomplish this by encoding for the enzyme ecdysteroid UDP-glycosyltransferase (EGT). Our studies showed that EGT activity was absent in the eulophid venom, which suggests that parasitic eulophids have developed a different and unique regulatory mechanism.



**PHARMACOLOGY OF SALIVA IN BLOOD-FEEDING HEMIPTERANS.** D. E. Champagne<sup>1</sup>, J.H. Valenzuela<sup>2</sup>, R. H. Nussenzveig<sup>2</sup>, and Jose M.C. Ribeiro<sup>2</sup>, <sup>1</sup>Department of Veterinary Science and Center for Insect Science, <sup>2</sup>Department of Entomology and Center for Insect Science, University of Arizona, Tucson, AZ. USA. No Abstract.

## IMMUNIZATION AGAINST A PEPTIDE VASODILATOR FROM SAND FLY SALIVA PROTECTS AGAINST LEISHMANIA TRANSMISSION IN MICE

Shoemaker, C.B., Siddiqui, A.A., Skelly, P.J., Lanzaro, G.C., Santos, A.H., Ribeiro, J.M., Titus, R.G., Dept. of Tropical Public Health, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115

Sand fly saliva contains multiple factors having distinct pharmacological activities. We have previously cloned and functionally expressed a cDNA that encodes one such factor, a highly potent peptide vasodilator called erythema inducing factor (EIF) that creates the characteristic "red spot" at the site of a bite. Characterization of EIF cDNAs from numerous different geographic isolates of sand fly indicate a high degree of polymorphism in the coding DNA with as much as 13 of 63 amino acid differences. Yet the vasodilatory specific activity of the recombinant proteins shows no significant differences.

The sand fly also transmits human leishmaniasis which is caused by the protozoan parasite, *Leishmania*, and salivary factor(s) are known to exacerbate infectivity, often by 10-100 fold. The parasites infect macrophages at the site of the fly bite from which they replicate and spread. The clinical manifestations of leishmaniasis range from self-healing cutaneous lesions to a potentially fatal visceral form depending upon the species of the parasite and the immunological response of the host. Recently we demonstrated that recombinant EIF, in addition to inducing erythema, can exacerbate experimental leishmaniasis. We then tested whether protein or naked DNA vaccination against this peptide will neutralize the exacerbative effects of saliva. Either immunization method induced both a humoral and cellular response to EIF. Immunized animals were then challenged by foot pad infections of  $10^5$  *Leishmania major* parasites in the presence of sand fly saliva. Two and four weeks post-infection, all vaccinated mice were found to have markedly reduced levels of parasites within their foot pads as compared to control animals. Immunization completely reversed the enhancing effect of saliva on *Leishmania* infectivity, even when the saliva contained EIF having many polymorphisms relative to the immunogen. These preliminary results suggest that EIF may be a target for vaccination against naturally transmitted *Leishmania*.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF NOXIUSTOXIN, A  $K^+$  CHANNEL BLOCKING-PEPTIDE FROM THE VENOM OF THE SCORPION *Centruroides noxius*

L.D. Possani, G.B.Gurrola, Martínez, F. and B. Becerril. Department of Molecular Recognition and Structural Biology, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Avenida Universidad, 2001, Apartado Postal 510-3, Cuernavaca, MEXICO 62271

Noxiustoxin (NTX), a 39 amino acid residue peptide was the first  $K^+$ -channel blocking-peptide described (Carlsberg. Res. Commun. 47:285-289, 1982), and was shown to recognize several types of  $K^+$ -channels with highly variable affinities (from micromolar to picomolar!). Important structural features of NTX were revealed after modification of the molecule by enzymatic and chemical cleavages, or by chemical synthesis of several peptides corresponding to the amino acid sequence of NTX, including the full length peptide, which were purified and assayed in binding experiments to membranes from rat brain synaptosomes using radio-labeled-NTX. The disulfide bridges of NTX were determined and its three-dimensional structure was modeled using computer graphic techniques. Also, the gene coding for NTX was synthesized by the recursive PCR method (Protein Engineering 5: 827-829, 1992). The PCR product was digested with *SalI* and *HindIII* and ligated into the expression vector pCSP105, kindly provided by Dr. Christopher Miller, and expressed in *E.coli* strain BL21, by means of the lambda lysogen containing the T7 RNA polymerase gene under the control of the *lacUV5* promoter, IPTG inducible. The recombinant protein was purified, digested with trypsin and the products were separated by HPLC. Approximately 1.3 mg/L of NTX was obtained and used for binding and displacement experiments. Experiments with edited genes, containing point mutations, are under way. The analysis of the results obtained thus far, suggests that the N-terminal segment of NTX, starting at the amino terminal and including part of the alpha-helix, is important for channel recognition.

Acknowledgements: Supported in part by grants from Howard Hughes Medical Institute (No.75191-527104), National University of Mexico (DGAPA-205893) and Consejo Nacional de Ciencia y Tecnología of Mexico to LDP.

**BEE AND ANT VENOM PROTEINS.** Donald Hoffman, Dept of Pathology and Laboratory Medicine, East Carolina University, Greenville, NC, USA.  
No Abstract.

# **INSECTICIDAL TOXINS FROM THE SPIDERS *DIGUETIA CANITIES*, *TEGENARIA AGRESTIS*, AND *FILISTATA HIBERNALIS*: CHEMICAL CHARACTERIZATION, MOLECULAR BIOLOGY, BIOLOGICAL ACTIVITY, AND POTENTIAL USES IN THE DEVELOPMENT OF NEW INSECTICIDES**

Robert M. Kral Jr., Karen J. Krapcho, Janice H. Johnson, Eric DelMar, Bradford C. VanWagenen, Irene V. Capuano, Christine K. Dunn, Forrest H. Fuller, Kathryn G. Eppler, and Terry K. Morgan

Three groups of insecticidal protein toxins have been identified from the venoms of the spiders *Diguetia canities* (Diguetidae), *Tegenaria agrestis* (Agelenidae), and *Filistata hibernalis* (Filistatidae). These toxins have potent insecticidal activity in a number of major crop pests, such as the tobacco budworm. Conversely, they are without demonstrable effects in a variety of mammalian *in vivo* and *in vitro* assays. The *Tegenaria* toxins have a high degree of sequence identity, but show distinct differences in potency toward different species of insects. The *Diguetia* toxins share a highly conserved N-terminus, but are more variable than the *Tegenaria* toxins. These two families of toxins are relatively small, with molecular masses ranging from 5500 to 7100, and appear to be highly structured molecules containing a number of disulfide bonds. They both cause persistent excitatory effects in insects, but their symptomologies are quite distinct and they may act at different target sites. The insecticidal *Filistata* toxins are much larger proteins, with molecular masses greater than 20,000. In insects, these toxins cause a gradually developing flaccid paralysis, frequently accompanied by a characteristic zone of discoloration. The potential uses of these toxins in recombinant bioinsecticides, and as tools for studying insect physiology, are discussed.

**OBSERVATIONS OF THE POTENTIAL COMMERCIAL USE OF  
ARTHROPOD VENOM MOLECULES. R. Todd. No Abstract.**

ALPHA SCORPION TOXINS BINDING ON RAT BRAIN AND  
INSECT SODIUM CHANNELS REVEAL DIVERGENT  
ALLOSTERIC MODULATIONS BY BREVETOXIN AND  
VERATRIDINE

Sandrine Cestele<sup>1</sup>, Rym Ben Khalifa<sup>2</sup>, Marcel Pelhate<sup>2</sup>, Herve  
Rochat<sup>1</sup> and Dalia Gordon\*<sup>1</sup>. <sup>1</sup>Faculty of Medicine Nord, I.F.R. Jean  
Roche, Laboratory of Biochemistry, Bd. Pierre Dramard, 13916  
Marseille Cedex 20, <sup>2</sup>Laboratory of Neurophysiology, University of  
Angers, 42045 Angers Cedex, France.

At least six topologically separated neurotoxin receptor sites have been identified on sodium channels, that reveal strong allosteric interactions among them. We have studied the allosteric modulation induced by veratridine, binding to receptor site 2, and brevetoxin PbTx-1, occupying receptor site 5, on the binding of alpha-scorpion toxins at receptor site 3, on three different neuronal sodium channels: rat brain, locust and cockroach synaptosomes. We used <sup>125</sup>I-AaH II, the most active alpha scorpion toxin on vertebrates and <sup>125</sup>I-LqhαIT, shown to have high activity on insects, as specific probes for receptor site 3 in rat brain and insect sodium channels. Our results reveal that brevetoxin generate three types of effects at receptor site 3: 1) Negative allosteric modulation in rat brain sodium channels; 2) Positive modulation in locust sodium channels and 3) No effect on cockroach sodium channel. However, PbTx-1 activates sodium channels in cockroach axon similarly to its activity in other preparation. Veratridine positively modulates both rat brain and locust sodium channels but had no effect on cockroach. The dramatic differences in allosteric modulations suggest structural differences in receptor sites for PbTx-1 and/or at the coupling regions with alpha scorpion toxins receptor sites in the different sodium channels, that can be detected by combined application of specific channel modifiers, and may elucidate the dynamic gating activity and the mechanism of allosteric interactions among various neurotoxin receptors.

## NON-ADRENERGIC NON-CHOLINERGIC (NANC) ACTION OF THE INDIAN RED SCORPION VENOM

M.C.E. Gwee, L.S. Cheah and <sup>1</sup>P. Gopalakrishnakone

Venom and Toxin Research Group, Departments of Pharmacology and  
<sup>1</sup>Anatomy, Faculty of Medicine, National University of Singapore, Kent  
Ridge, SINGAPORE 0511.

We have investigated the possibility that the venom (RSV) from the Indian red scorpion *Mesobuthus tamulus* can elicit, in the rat isolated anococcygeus muscle (Acm), relaxant responses mediated via NANC nerve stimulation since RSV can cause generalised peripheral nerve depolarisation with consequent enhancement of neurotransmitter release and nitric oxide (NO) is strongly implicated as the NANC (inhibitory) neurotransmitter mediating relaxant responses of the Acm. The Acm was mounted under 1g tension in Krebs solution containing 5 $\mu$ M phentolamine, aerated with 5% CO<sub>2</sub> in O<sub>2</sub> and maintained at 37°C. The Acm was precontracted with 5 $\mu$ M carbachol and responses were recorded isometrically: RSV 1.5 $\mu$ g/ml produced a fairly marked relaxation (maximum:  $27.2 \pm 2.5\%$ ,  $n=8$ ;  $4 \pm 0.4$ min) of the Acm tone which then gradually recovered to  $90.7 \pm 2.1\%$  of the original peak tension over  $22.5 \pm 0.8$ min; addition of a second dose (1.5 $\mu$ g/ml) of RSV did not change the muscle tone, but relaxations ( $79.8 \pm 3.7\%$ ;  $n=4$  and  $27.8 \pm 6.4\%$ ;  $n=4$ , respectively) in muscle tone were obtained when either 1 $\mu$ M sodium nitroprusside or 250 $\mu$ M L-arginine was then added. Prior treatment of the Acm with either 50 $\mu$ M N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) or 2 $\mu$ M tetrodotoxin (TTx) blocked its relaxant responses to RSV and to electrical field stimulation (20-30V, 10Hz x 10sec, 1msec pulse width, every 2min). Our results suggest that the RSV-induced relaxant responses of the Acm is likely to be NO mediated, presumably as a consequence of NANC nerve depolarisation by RSV.

Acknowledgement: Supported by grants (RP870357 and RP890321) from NUS.



## ACUTE PHASE RESPONSE FOLLOWING SCORPION STING IN CHILDREN

S. Sofer. Pediatric Intensive Care Unit, Soroka Medical Center and  
Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Children with scorpion envenomation (SE) may present with signs of systemic inflammatory response which include tachycardia, tachypnea, hypothermia or hyperthermia and leukocytosis. This may lead in some cases to secondary organ failure and death, which may be caused partially by activation of cytokines and release of interleukins (IL). In order to test this theory, IL-6 was determined (by ELISA) in serum of 10 children admitted to the Pediatric Intensive Care Unit with severe SE.

Measurements were taken on arrival and at 12 and 24 h. IL-6 was markedly elevated in 8/10 children on arrival (average 33 pg/ml) and approached normal levels at 12 and 24 h (average 9 pg/ml). All IL-6 values were above those of controls (4 pg/ml each). These results may imply that acute phase response and release of interleukins play an important role in the pathophysiology of SE. Human and experimental animal studies are required to verify this theory. The production and release of other pro-inflammatory cytokines following SE, needs to be studied. If cytokines is found to play a considerable part in pathophysiology this may have an impact on therapy.

Mechanisms of Secretory Discharge Stimulated by  
Scorpion Venom

Fletcher, P.L., Fletcher, M.D., L.K. Fainter, and S.M.  
Lucas. E.C.U. School of Medicine, Greenville, NC 27858  
and

Instituto Butantan, Sao Paulo, Brazil

Venom from Brazilian scorpions of the genus Tityus that are known to be dangerous to man (T. serrulatus, bahiensis, and stigmurus) contain protein toxins that are characterized to act on specific ion channels in both excitable and non-excitable cells. We are studying these mechanisms in both excitable (cerebral  $P_2$  synaptosomes) and non-excitable (pancreatic acinar) cells. Experiments with  $Na^+$ ,  $K^+$ , and  $Ca^{++}$  ions reveal different mechanisms for the stimulation systems for each cell type. The protein toxins (of Mw 6200 kD) were found to possess subtle structural variations that exert marked changes in the ionic requirements for activation of secretory mechanisms. The most striking changes appear in the  $\alpha$ -scorpion toxin (toxin IV-5) that, in contrast to the  $\beta$ -scorpion toxins ( $\Gamma$  and III-8), act by a  $Ca^{++}$ -independent mechanism. We will present our findings in recent studies of these mechanisms in the context of molecular structural characteristics.

ISOLATION AND CHARACTERIZATION OF THE GENES ENCODING  
GAMMA TOXIN OF THE BRAZILIAN SCORPIONS *Tityus serrulatus*,  
*Tityus bahiensis* AND *Tityus stigmurus*.

B. Becerril, M. Corona and L.D. Possani. Department of  
Molecular Recognition and Structural Biology, Instituto  
de Biotecnología, Universidad Nacional Autónoma de  
México, Apartado Postal 510-3, Cuernavaca-62271, México.

The genes encoding toxin gamma from the scorpions *Tityus serrulatus*, *T. stigmurus* and *T. bahiensis* were amplified from genomic DNA by means of PCR using synthetic oligonucleotides designed from the reported cDNA sequence of gamma toxin from *T. serrulatus*. The analysis of the nucleotide sequence of these genes revealed the presence of introns of 475, 474 and 464 base pairs in the genes of *T. serrulatus*, *T. stigmurus* and *T. bahiensis* respectively, which interrupt the region that encodes the signal peptide of the respective precursor toxin. A distinctive feature of gamma toxin from *T. stigmurus* concerns the presence of an additional glycine residue at the amino end of the mature toxin compared with the other two gamma toxins above mentioned. The interpretation of the mechanism for the processing at the amino terminus of this peptide will be discussed. Finally a comparison of the intron boundary sequences of the gamma toxin genes with the ones from other arachnid genes will also be presented.

Acknowledgments: This work was supported in part by grants of the Howard Hughes Medical Institute (No.75191-527104), National University of Mexico (DGAPA-205893) and Consejo Nacional de Ciencia y Tecnología of Mexico to LDP.

## SELECTIVE MODIFICATION OF SODIUM CHANNEL INACTIVATION BY VERSUTOXIN AN AUSTRALIAN FUNNEL-WEB SPIDER TOXIN

<sup>1</sup>G.M. Nicholson and <sup>2</sup>T. Narahashi

<sup>1</sup>Department of Biochemistry & Physiology, University of Technology, Sydney, NSW 2007, Australia, <sup>2</sup>Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611, USA

Venom from the Australian funnel-web spiders *Hadronyche versuta* and *Atrax robustus* have previously been shown to produce repetitive action potentials in nerve fibres leading to spontaneous transmitter release at motor and autonomic nerve endings. This action may involve an alteration to voltage-gated sodium channel function. To assess this possibility the effects of versutoxin, a novel 42 amino acid peptide from the venom of the Australian Blue Mountains funnel-web spider *H. versuta*, was investigated on ionic currents in acutely dissociated rat dorsal root ganglion cells. Whole-cell patch clamp experiments revealed that versutoxin (VTX), at concentrations up to 2  $\mu$ M, had no effect on potassium currents or tetrodotoxin-resistant sodium currents. In contrast VTX produced a concentration-dependent slowing or removal of tetrodotoxin-sensitive sodium current (TTX-S  $I_{Na}$ ) inactivation when applied via the external but not the internal bathing solution. The prolonged steady-state  $I_{Na}$  seen in the presence of VTX was maintained during prolonged depolarising pulses greater than 200 ms. At higher test potentials, however, there was a reduction in the amplitude of the steady-state  $I_{Na}$  which may reflect a voltage-dependent dissociation of the toxin from the extracellular binding site. Unlike  $\beta$ -scorpion toxins, VTX did not significantly alter the timecourse of TTX-S  $I_{Na}$  tail currents. Examination of the steady-state inactivation curve ( $h_{\infty}$ ) of the sodium current revealed that 30 nM VTX produced a significant 7 mV hyperpolarising shift in the voltage dependence of  $h_{\infty}$  and that there was a significant non-inactivatable  $I_{Na}$ . This non-inactivating component ( $14 \pm 2\%$  of maximal  $I_{Na}$ ) was present at prepulse potentials more depolarized than -40 mV, potentials which normally inactivate all TTX-S sodium channels. Finally, there was an increase in the rate of recovery from channel inactivation in the presence of VTX. In conclusion these selective actions of VTX on sodium channel gating, particularly the voltage-dependent slowing of channel inactivation and the faster rate of recovery from inactivation, are similar to those of  $\alpha$ -scorpion toxins. They also support the model of a two state inactivation process proposed by Strichartz and Wang (1986) to explain the modification of sodium channel gating by  $\alpha$ -scorpion toxins. G.R. Strichartz and G.K. Wang (1986) *J. Gen. Physiol.* 88, 413-435.

## CIGUATERA (FISH POISONING): PROGRESS AND PERSPECTIVES

Richard J. Lewis

Queensland Department of Primary Industries, QABC, Gehrmann Laboratories,  
The University of Queensland, QLD 4072, Australia

Ciguatera, a pleomorphic syndrome consisting of a range of gastrointestinal, neurological and cardiovascular signs and symptoms, follows the consumption of warm-water marine fish that have become contaminated with the ciguatoxin class of polyether toxins. The disease is rarely fatal and the severity and duration of illness can be markedly reduced with intravenous mannitol. The ciguatoxins (CTX) arise from the oxidative biotransformation of gambiertoxin(s) produced by certain strains of the benthic dinoflagellate, *Gambierdiscus toxicus*. Following blooms of *G. toxicus*, these toxins are concentrated in fish through marine food chains to levels that affect human health. Factor(s) influencing such bloom formation are unclear but subtle environmental effects may explain the patchy and unpredictable nature of ciguatera. CTX-1, the most potent sodium channel toxin known, is the major toxin in ciguateric carnivorous fish in the Pacific. Levels of 0.1 ppb ( $10^{-10}$  mole CTX-1/kg) and above can result in human poisoning. The less potent gambiertoxins are the major toxins found in Pacific herbivorous fish. Other toxins produced by benthic dinoflagellates, including okadaic acid and maitotoxin, have no proven role in ciguatera. Recent studies indicate that the major toxin in ciguateric carnivorous fish from the Caribbean Sea and Indian Ocean differ from Pacific ciguatoxins. It is uncertain if *G. toxicus* is the origin of these toxins. The mouse assay is presently widely used to assess levels of ciguatoxin in fish extracts. Other *in vivo* assays, including the chicken, mongoose, mosquito, brine shrimp and diptera larvae assays, are less widely used. *In vitro* cell-based assays that measure the effects of ciguatoxin-induced sodium channel opening or the inhibition of [ $^3\text{H}$ ]-brevetoxin binding are more sensitive than *in vivo* methods and have the potential to replace these assays. Analytical detection methods are also under development. The ciguatoxins do not possess a distinctive chromophore but do possess a relatively reactive primary hydroxyl through which a label can be attached prior to detection by HPLC/fluorescence or HPLC/ion-spray mass spectrometry. Antibody-based assays hold much potential as cost-effective screens for ciguateric fish but presently suffer from a lack of specificity and sensitivity. A major advance in the management of ciguatera will come when such screens are validated and commercially available.

## ASSESSMENT OF METHODS FOR THE DETERMINATION OF CIGUATOXINS

<sup>1</sup>R.W. Dickey, <sup>2</sup>R.L. Manger, <sup>3</sup>M.A. Poli, <sup>4</sup>F.M. Van Dolah, <sup>5</sup>J.A. Delgado-Arias, <sup>6</sup>S.M. Musser, <sup>5</sup>D.G. Baden, <sup>2</sup>J. Hungerford, <sup>5</sup>K.S. Rein, <sup>2</sup>S. Lee, <sup>4</sup>T. Leighfield, and <sup>1</sup>H.R. Granade <sup>1</sup>FDA, GCSL, Dauphin Island, AL 36528, <sup>2</sup>FDA, SPRC, Bothell, WA 98021 <sup>3</sup>USAMRID, Fort Detrick, Frederick, MD 21702, <sup>4</sup>NMFS, SFSC, Charleston, SC 29422, <sup>5</sup>NIEHS, Univ. of Miami, Miami, FL 33149, <sup>6</sup>FDA, IBB, Washington D.C. 20204

Analysis of seafoods for ciguatoxin contamination has long relied upon the mouse bioassay. For reasons both substantive and perceptual the replacement of whole animal testing is desirable. In a collaboration involving 6 laboratories the mouse bioassay, LC-MS and two *in vitro* assays were used for the determination of ciguatoxins from finfish tissues. The *in vitro* assays, brevetoxin receptor binding competition and ouabain-veratridine dependent cytotoxicity, measure sodium channel specific activity. Using PbTx-3, P-CTX3C, and C-CTX1 as standards in the analysis of 2 control and 11 barracuda tissue specimens recovered from 2 ciguatera outbreaks, the *in vitro* assays provided greater sensitivity and specificity than did mouse bioassay. Qualitatively, results from all laboratories identified 5 toxic specimens from the battery of 13 test samples. Detection of low level activity in 5 additional specimens was not consistent between laboratories. In the binding assays, interlaboratory within-sample quantitative differences ranged up to one order of magnitude and may reflect systematic error. Correcting for the presumed systematic error reduced the total number of toxic specimens to 6 with 4 containing significantly higher levels of activity. Mass spectrometric analysis of the test samples identified C-CTX1, a Caribbean form of ciguatoxin ( $MH^+$  1123.6), only in the 4 specimens containing higher activity. Mean C-CTX1 concentrations estimated by binding assays ranged from 1.5 to 21.8 ppb. The cytotoxicity assay provided more conservative estimates ranging from 1.2 to 6.0 ppb for the same specimens. Acute responses by mouse bioassay were observed in only 2 of the 6 toxic specimens. Subacute responses were observed in 5 additional test samples including those identified by *in vitro* assays. The study documents significantly improved capabilities for the screening and confirmation of ciguatera toxins in finfish.

## ANALYTICAL MEASUREMENT OF CIGUATOXIN LEVELS IN FISH TISSUE USING MASS SPECTROMETRY

Steven M. Musser<sup>1</sup>, H. Ray Granade<sup>2</sup> and Robert W. Dickey<sup>2</sup>

<sup>1</sup>Center for Food Safety and Applied Nutrition, FDA, Washington, DC 20204

<sup>2</sup>Gulf Coast Seafood Laboratory, FDA, Dauphin Island, AL 36528

The development of instrumental methods for the identification of ciguatoxin (CTX) in contaminated fish tissue are complicated by several factors, among which are the lack of a chromophore or easily derivatized functionality, limited volatility and the low detection limits necessary for relevance to seafood safety. While *in vivo* and *in vitro* bioassays can provide sufficient sensitivity to detect and estimate the amount of CTX in tissue, these methods cannot equal the degree of specificity and precision of instrumental methods. Because there are at least two isomers of CTX as well as other toxins which may be present in the sample, a method which could provide specificity in addition to sensitivity would be useful. Since any analytical method for CTX would require HPLC as a chromatography step, the recently developed HPLC/MS ionization technique known as atmospheric pressure ionization (API) was evaluated as a means of confirming and quantitating CTX in contaminated fish tissue. Introduction of either Pacific CTX (P-CTX) or Caribbean CTX (C-CTX) into the ion source via HPLC produced primarily the protonated species of CTX, P-CTX (1111) and C-CTX (1123). In addition to the molecular ion, several intense ions corresponding to consecutive losses of water from the parent compound were present in the mass spectrum. Application of LC/API/MS to the determination of CTX in fish samples provides a reliable method for accurately confirming both the type (Pacific or Caribbean) and amount of CTX present in the sample. The method distinguishes non-CTX toxin samples from P-CTX or C-CTX by mass, fragmentation pattern and HPLC retention time, thereby providing a high degree of specificity. In fish samples known to contain CTX at toxic levels, by bioassay, the presence and amount of CTX could be determined in all but the lowest (< 1 ppb) toxin containing samples.

SUBMICROMOLE STRUCTURE ELUCIDATION USING  
MICRO INVERSE DETECTION -- APPLICATIONS TO MARINE  
TOXIN STRUCTURE ELUCIDATION

G.E. Martin<sup>1</sup>, R.C. Crouch<sup>1</sup>, R.W. Dickey<sup>2</sup>, H.R. Granade<sup>2</sup>, and S.M. Musser<sup>3</sup>

<sup>1</sup>Bioanalytical Sciences NMR Laboratory, Burroughs Wellcome Co., Research Triangle Park, NC 27709, <sup>2</sup>Gulf Coast Seafood Laboratory, US FDA, Dauphin Is., AL 36528, <sup>3</sup>Instrumentation and Biophysics Branch, FDA, Center for Food Safety and Applied Nutrition, Washington, DC 20204

NMR detection sensitivity has been improved dramatically during the last several years by the development of inverse-detected heteronuclear shift correlation experiments (e.g. HMQC, HMBC, etc.) coupled with micro inverse probes. Employed in tandem, these developments have made total structure elucidation with samples consisting of 1  $\mu$ mole quite straightforward. Smaller samples, i.e. those consisting of  $\sim 0.25$   $\mu$ mole, while much more challenging, can still be undertaken with considerable confidence of success. Samples consisting of 0.1  $\mu$ mole or less remain a difficult undertaking with no guarantees of success, especially when molecules become relatively large and/or spectrally complex. Marine toxins, because of their scarcity, often fall into one of the latter two categories. Beginning with an  $\sim 1$   $\mu$ mole brevetoxin-3 as an example, a micro detection strategy for the total assignment of both the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra will be presented which relies on the concerted utilization of TOCSY, HMQC, HMBC, IDR-(Inverted Direct Response)-HMQC-TOCSY, and ROESY spectra. The strategy to be presented can also be readily employed to unequivocally establish new structures. Additional illustrations of these techniques will be presented using okadaic acid as an alternative model compound. Finally, preliminary work completed using a sample of  $<0.1$   $\mu$ mole of Caribbean ciguatoxin (C-CTX1) will also be presented. HMQC spectra acquired using conventional 3 mm micro inverse detection will be compared to those obtainable using specialized NMR micro cells. Vastly superior data can be acquired in  $<50\%$  of the instrument time using the latter approach. Limited HMBC correlations from the methyl groups of C-CTX1 were also accessible using specialized NMR micro cells in a reasonable period of time. Structural implications which can be drawn from these data and a comparison of the homology of C-CTX1 to the corresponding Pacific toxin will also be presented.



**THE DISCOVERY OF DOMOIC ACID AS A NEW MARINE TOXIN.**  
Jeffrey Wright, National Research Council of Canada.-No Abstract

## SEAFOOD TOXINS: OUTBREAKS, RESEARCH, AND MANAGEMENT

S. Hall

Office of Seafood, U. S. Food and Drug Administration HFS-426,  
Washington, D. C. 20204 U. S. A.

The biomass we consume as seafood starts out as unicellular marine plankton, transformed through one or more steps into fish or shellfish. Unfortunately, some species of plankton produce potent natural toxins that accumulate in seafood and can cause death or serious illness in human consumers. To manage the problem we need to understand the nature of the toxins and the organisms that produce them. Such information, together with experience from recent outbreaks, is helping to guide the development of more cost-effective approaches for ensuring seafood safety.

DEVELOPMENT OF A PROTEIN PHOSPHATASE BASED ASSAY FOR THE DETECTION OF PHOSPHATASE INHIBITORS IN CRUDE WHOLE CELL/ANIMAL EXTRACTS. Richard E. Honkanen<sup>1</sup>, Faith R. Caplan<sup>2</sup>, Gregory M. L. Patterson<sup>2</sup> and <sup>1</sup>Jennifer Abercrombie. <sup>1</sup>Department of Biochemistry and Molecular Biology, University of South Alabama, Mobile, AL 36688, <sup>2</sup>Department of Chemistry, University of Hawaii, Honolulu, HI.

Diarrhetic shellfish poisoning (DSP) is a serious and globally widespread phytoplankton-related seafood illness. Although rarely life threatening, DSP causes incapacitating diarrhea and vomiting with no known medical treatments. Phytoplankton producing DSP-toxins have been identified in temperate coastal waters world wide, and their numbers may be increasing as a result of eutrophication. The toxic effects of the major DSP-toxins (okadaic acid and 35 methyl okadaic acid) apparently originate from their inhibitory activity against a family of structurally related serine/threonine protein phosphatases (PSPases). In particular, the inhibition of essential PSPases (e.g. PP1 and PP2A), has catastrophic consequences to most eukaryotic cells. Exploiting the unique pharmacological property of the DSP toxins as phosphatase inhibitors, we have developed an assay (PP2A-assay) capable of detecting both okadaic acid and dinophysistoxin-1 in pg amounts. This assay employs purified PP2A, which has an extremely high affinity for both DSP-toxins. This provides the PP2A-assay a level of sensitivity comparable to, or surpassing, that of most monoclonal antibody probes. The PP2A-assay was employed to detect the presence of PSPase inhibitors in extracts of shellfish, sponges, and cultured cyanophytes. The findings from these studies indicate that phosphatase inhibitors may be more widely distributed in the marine environment than previously believed. In addition, the findings suggest that the PP2A-assay can be developed into a rapid and relatively simple method for detecting PSPase inhibitors in crude cell extracts produced from a variety of sources. This work was funded in part by a grant (NA47FD0429) from the National Oceanic and Atmospheric Administration.

**ANTIBODIES TO MAITOTOXIN ELICITED BY IMMUNIZATION WITH  
TOXIN FRAGMENT CONJUGATES. Gary Bignami - No Abstract**

## EFFECTS ON FISHERIES AND HUMAN HEALTH LINKED TO A TOXIC ESTUARINE DINOFLAGELLATE

J.M. Burkholder and H.B. Glasgow Jr. North Carolina State  
University, Raleigh, NC 27965-7612 U.S.A.

The estuarine dinoflagellate *Pfiesteria piscicida* gen. & sp. nov. (Steidinger et al.) has been implicated as the causative agent of ca. 50% of the major fish kills ( $10^3$  to  $10^9$  fish) in the Albemarle-Pamlico Estuarine System, NC. *P. piscicida* is stimulated by fresh fish secreta, and it was lethal to all 19 species of fish bioassayed in culture. This dinoflagellate produces exotoxin(s) that create both neurotoxic water and aerosols. Culture filtrate induces formation of open ulcerative sores, hemorrhaging, and death of finfish and shellfish. Human exposure to culture aerosols has been linked to narcosis, respiratory distress, nausea, eye irritation (hours to days); other autonomic nervous system dysfunction (high localized perspiring, erratic heart beat [weeks]); and central nervous system dysfunction (sudden rages, other erratic behavior [hours - days]; reversible cognitive impairment, short-term memory loss [weeks]). Elevated hepatic enzyme levels and high P excretion suggested hepatic and renal dysfunction (weeks); easy infection and low counts of several T cell types indicated immune system suppression (months to years). *P. piscicida* is euryhaline and eurythermal, and is stimulated by organic P enrichment. Pfiesteria-like dinoflagellates have been tracked to fish kill sites in eutrophic estuaries from Delaware Bay through the Gulf Coast. Our data point to a critical need to characterize their chronic effects on human health as well as fish recruitment, disease resistance, and survival.

## IDENTIFICATION OF A SODIUM CHANNEL TOXIN FROM A CARIBBEAN BENTHIC DINOFLAGELLATE

G. Escalona de Motta, A. L. Rivera Rentas, J. A. Mercado, T. Tosteson<sup>\*</sup>, and I. González<sup>\*</sup>. University of Puerto Rico, Institute of Neurobiology and Department of Biology, San Juan, PR 00901 and <sup>\*</sup> Department of Marine Sciences, Mayagüez, PR 00680 .

*Ostreopsis lenticularis* cells treated with acetone before methanol extraction showed a tenfold increase in mouse toxicity and an HPLC chromatogram with a major peak in the 5 min region termed by us the ostreotoxin-3 (OTx-3) peak. Intracellular recording experiments demonstrated that OTx-3-enriched extracts depolarized frog skeletal muscle and decreased the amplitude of endplate potentials without affecting the nerve impulse. This effect was partially blocked by 10  $\mu$ M TTx and was totally blocked by 5  $\mu$ M  $\mu$ -Conotoxin GIIIA ( $\mu$ -CTx). Using the whole-cell configuration of the patch clamp technique we identified two pharmacologically different populations of voltage dependent sodium channels in chick embryo neurons: TTx-sensitive channels that produced nearly 80% of the current and a minor fraction of  $\mu$ -CTx-sensitive channels that produced approximately 20%. These sodium currents were affected by OTx-3 extracts. The most toxic one, 223-4B, shifted the voltage dependence of both current activation and inactivation but had its major effect on the inactivation curve. There it caused a shift of 30 mV to the negative of the  $V_{50\%}$  without any change in the curve steepness. In the presence of  $\mu$ -CTx GIIIA, the remaining sodium current was not altered by the OTX-3 extract. Thus, the myotoxic actions of *O. lenticularis* extracts may be explained by the presence of a sodium channel toxin that recognizes a precise subclass of voltage-gated,  $\mu$ -CTx GIIIA-sensitive sodium channels.

## ALKALOID NEUROTOXINS FROM CARIBBEAN SPONGES OF THE GENUS AGELOS: EFFECT ON NEURONAL MEMBRANE CHANNELS

A. L. Rivera-Rentas, R. Rosa, A. D. Rodríguez\*, W. Silva\*\* and G. Escalona de Motta. University of Puerto Rico Institute of Neurobiology and Departments of Biology and \*Chemistry, San Juan PR 00901, and Universidad Central del Caribe School of Medicine \*\*Department of Pharmacology, Bayamón, PR 00958.

Compounds of the  $C_{11}N_5$  family found in tropical *Agelas* sponges have been shown to be active on muscle and nerve receptors. Clathrocin (CLA) and dibromosceptrin (DBS) are two of these compounds which differ in structure, CLA being a monomer and DBS a dimer; and in bromide substitutions which range from none (CLA) to four (DBS). Voltage clamp experiments on frog muscle showed that both agents altered voltage dependent ionic currents, CLA causing an early potentiation followed by total inhibition and DBS exerting an immediate current blockade. Their effects on ionic channels were examined using CLA and DBS in radioligand binding studies which showed that DBS inhibited nearly 90% of  $^3H$ -saxitoxin (STx) binding to rat brain synaptosomes while CLA inhibited only 30%. As these results suggest an action on sodium channels, we studied the effect of CLA and DBS on membrane sodium currents of sympathetic neurons using the whole-cell patch clamp technique. Though low CLA concentrations produced a potentiation, both agents decreased peak current amplitudes by an average of 27% for CLA and 40% for DBS. Neither altered the voltage dependence of current activation but, in experiments done applying two-pulse protocols to measure current inactivation parameters, CLA caused only a slight positive shift on the voltage dependence whereas DBS caused a negative shift of nearly 20 mV on the  $V_{50\%}$  value of the inactivation curve without affecting curve steepness. In terms of the time course of inactivation, DBS increased  $\tau$  twice as much as CLA. DBS also delayed the recovery from inactivation or reactivation and increased the time for half recovery by 2 ms. Thus, CLA and DBS are novel sodium channel agents which bind to a site that displaces STX binding. Their functional specificity, however, appears to be different; DBS, the dimer with four bromide substitutions, acting at the voltage dependence of inactivation, and CLA, the monomer without bromine substitutions, directed toward the channel ionic conductance.

The Sea anemone Anemonia sulcata is a rich source of biologically active polypeptides.

Lászlo Béress.

Institute of Toxicology, Christian-Albrechts-University, Brunswikerstr. 10 D24105  
Kiel, Germany.

Since our first publication appeared (1) the sea anemone Anemonia sulcata became a wellknown natural source of supply of biologically active polypeptides. Up to now five toxins ATX I, II, III, IV (2,3) and AS V (4), several polyvalent protease inhibitors(5), an elastase inhibitor(6), two blood pressure depressive polypeptides(7) and very recently also peptides, inhibiting competitively the binding of  $^{125}\text{I}$  dendrotoxin to rat brain membranes, blocking the voltage sensitive  $\text{K}^+$  channels(8), have been isolated from it. It could be demonstrated, that only the final use of HPLC on a RP-C18 reversed phase column enables the total purification of the Anemonia sulcata toxins because each sea anemone toxin contain several isotoxins, polypeptides differing only by one amino acid in the sequence (9). The sea anemone toxins ( especially the toxin II of Anemonia sulcata ATX II) became very important tools in the neurophysiological and pharmacological research (3), their structure function relationship is investigated (10). The purification techniques elaborated for isolation of the Anemonia sulcata polypeptides can be easily applied for isolation of toxins from other natural resources.

#### References:

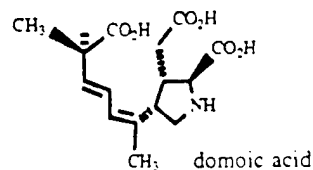
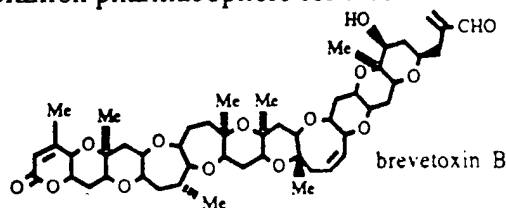
- 1) Béress, L., Béress, R. Kieler Meeresforschungen 27, 117-127 (1971).
- 2) Béress, L., Béress, R., Wunderer, G. Toxicon 13, 359-367 (1975).
- 3) Béress, L. In 'Poisonous and Venomous Marine Animals of the world' 2nd edn. (B.W. Halstead) Darwin Press Princeton NY 1988, pp. 150-161.
- 4) Schweitz, H., Vincent, J.P., Barhanin, J., Frelin, C., Linden, G., Hugues, G., Lazdunski, M. Biochemistry 20, 5245-5252 (1981)
- 5) Fritz, H., Brey, B., Béress, L. Hoppe-Seyler's Z. Physiol. Chem. 353, 19-30 (1972).
- 6) Kolbenbrock, H., J. and H. Tschesche. Biol. Chem. Hoppe Seyler 368, 93-99.
- 7) Béress, L., Leimenstoll, A., Horaczynski, H., Henschen, A., Walsh, K.A. . In 'Proc. of the 7<sup>th</sup> USSR-FRG Sym. of Peptides and Proteins' Dilizhan (1989).
- 8) Schweitz, H. et al., unpublished result.
- 9) Wachter, E., Klostermann, G., Binder, A., Béress, L. In 'Receptors and Ion Channels' (Eds. Ovchinnikov, Y.A. and Hucho, F.) de Gruyter 1987 pp.313-322
- 10) Norton, R.S. Toxicon 29, 1051-1084 (1991)



## CONFORMATIONAL ANALYSIS OF MARINE NEUROTOXINS AND A HYPOTHESIS CONCERNING THE COMMON PHARMACOPHORES

Kathleen S. Rein, University of Miami, Rosenstiel School of Marine and Atmospheric Science, Division of Marine and Atmospheric Chemistry, Miami, FL 33149

A number of marine organisms produce potent neurotoxins. These toxins typically exert their effects by binding to a membrane bound receptor. The receptor may have an endogenous ligand, or may be an orphan receptor, meaning that there is no known function and no known endogenous ligand. We have been studying ligand-receptor interactions for two classes of marine neurotoxins. The polyether ladder compounds, brevetoxins and ciguatoxin, bind to an orphan receptor which activates the voltage-gated sodium channel. Domoic acid belongs to a larger family of compounds known as the kainoids which may be regarded as conformationally restricted glutamate analogs. This family of compounds binds to and activates a ligand-gated ion channel, for which the endogenous ligand is glutamate. Both classes of compounds have been generally regarded as conformationally rigid. Our studies have revealed that both classes of compounds possess a fair degree of conformational flexibility. The kainoids in particular, may adopt a number of distinct ring conformations. A conformational bias is induced in the pyrrolidine ring by the C4 substituent and this bias is translated into the binding affinity. Through a combination of synthetic modification of the natural toxins, evaluation of the effects of these modifications on binding affinity and activity, and conformational analysis, we have developed a hypothesis concerning the common pharmacophore for these two classes of toxins.



**Caribbean *G. Toxicus*: Purification and Characterization of Ciguatera Related Seafood Toxins.**

Peter D.R. Moeller, Sharon E. Davis, Stewart M. Knoepp and Bernie A. Lanoue

Marine Biotoxins Program  
NOAA Southeast Fisheries Science Center  
217 Fort Johnson Road, Charleston, SC 29412, USA

Maitotoxin (MTX) and ciguatoxin (CTX) congeners are involved in ciguatera seafood poisoning. These toxins have proven difficult to isolate and purify in substantial quantities. This is due in large part to copious amounts of pigments in the crude extracts, lack of stability of these toxins under normal chromatographic conditions, as well as the variability of their chemical reactivity due to changes in chemical environment. In pursuit of isolating and characterizing several of these toxins on a preparative scale, mass cultures of a Caribbean strain of *G. toxicus* were grown to provide enough extract to supply the needs for structural determination as well as for pharmacological and toxicological studies at the National Marine Fisheries Service. We wish to present research in the areas of new purification methodology utilizing modified iso-electric focusing techniques, mass spectrometric protocols and data as well as nmr spectra of several MTX congeners. Comparison to known toxins of similar biological activity is also discussed.

## STRAIN-DEPENDENT PRODUCTION OF TOXINS BY BENTHIC DINOFLAGELLATES

M.J. Holmes

Bioscience Centre, National University of Singapore, Singapore.

The majority of benthic dinoflagellates produce toxins. However, the types of toxins produced vary considerably depending not only upon the species but also upon the isolate cultured. The benthic dinoflagellate *Gambierdiscus toxicus* in particular has been the subject of numerous studies because of its role in producing the precursor(s) of the ciguatoxins that accumulate in fish to cause ciguatera. The toxin profiles of three strains of *G. toxicus* (FP, NQ1 and WC1/1) grown under identical culture conditions differ markedly with ciguatoxin precursors detected only from the WC1/1 strain. In contrast, all three strains produce maitotoxins. However, the FP and NQ1 strains produce large maitotoxins (>3,000 molecular weight) whereas the WC1/1 strain produces a small maitotoxin (molecular weight of 1,060 for the disodium salt). Strain-dependent toxin production also occurs with the benthic dinoflagellate *Coolia monotis*. *C. monotis* was previously thought to be a non-toxic species. However, a strain of *C. monotis* isolated from Platypus Bay in Australia was found to produce a new toxin that was named cooliatoxin. Cooliatoxin has a molecular weight of 1,062 for the sodium salt and an intraperitoneal LD<sub>50</sub> of 1 mg.kg<sup>-1</sup> in mice. It induces hypothermia and respiratory failure in mice after a pronounced delay period during which there are no obvious signs of intoxication. These bioassay signs have not been reported from toxic fish extracts and therefore there is no evidence to suggest that cooliatoxin accumulates in fish to cause human poisoning.

## THE COMPUTER ANALYSIS OF AMINO ACID SEQUENCES OF TOXIC AND NONTXIC PHOSPHOLIPASES A<sub>2</sub>

<sup>1</sup>A.N.Vagin, <sup>1</sup>O.N.Vagina and <sup>2</sup>I.I.Parilis

<sup>1</sup>Tashkent State University, Tashkent, Uzbekistan

<sup>2</sup>Institute of Biochemistry, Tashkent, Uzbekistan

The primary structures of 68 toxic and nontoxic phospholipases A<sub>2</sub> from "Swiss-Prot.Rev.14" protein bank have been studied. We designed computer programs to compare the amino acid sequences of above enzymes. The hexapeptide YGCYCG which was common for all phospholipases A<sub>2</sub> and absent in the other 15400 proteins of the bank was detected using these programs. Pancreatic phospholipases A<sub>2</sub> from different sources showed high level of similarity with each other: there were fragments YGCYCGLGGSGTPVD, DNPYT, FICNCDR, AAICFSK which were found only in pancreatic phospholipases among the proteins of the bank. The presence of CYG site and the fragment with "mistakes" (the length of 19 amino acid residues) in neurotoxic phospholipases and their absence in pancreatic phospholipases were shown. The obtained results maybe useful in the determination of the "toxic" site in the neurotoxic phospholipases A<sub>2</sub>. The developed programs can be applied in the detection of specific fragments in other classes of proteins. We thank Prof. Khamidov D.K. for offered opportunity of using the information from the bank.

## COMPARISON OF *VARANUS GRIZEUS* PHOSPHOLIPASE A WITH VENOM ENZYMES

A. Khafizov, I.I. Parilis, E. Kazanov, M. Akramov, R..S. Salikhov,  
D. Kh. Khamidov  
Institute of Biochemistry, Tashkent, Uzbekistan

All known phospholipases  $A_2$  are classically separated into two classes on the basis of some structural differences: group I comprises phospholipases from pancreatic juice, elapids and group II - from many crotalids. Lizard enzymes with similar bee enzyme form a specific class O.

A protein with phospholipase A activity was isolated from the secret of grey monitor *Varanus grizeus* using Ultrigel AcA-54 gel-filtration. The enzymatic activity was measured by the egg yolk clotting method. This protein has an apparent MM of 13,000 by sodium dodecyl sulfate PAAG electrophoresis. Its isoelectric point is basic (pI 10.0).

By means of a computer method we derived the amino acid composition of the purified enzyme which was compared to that of more than 50 phospholipases from different venoms. According to these data a structural similarity of *Varanus grizeus* protein to other phospholipases  $A_2$  decreases as follows: *H. suspectum*, *H.h. horridum*, *A. mellifera*, crotalids (group II), elapids (group I).

# GENOMES OF *VIPERIDAE* SNAKES CONTAIN *ARTIODACTYLA* SPECIFIC ART-2 RETROPOSON

D. Kordiš and F. Gubenšek

Department of Biochemistry and Molecular Biology, Jožef Stefan Institute,  
Jamova 39, 61111 Ljubljana, Slovenia

Comparison of highly conserved intron sequences of *Viperidae* venom PLA<sub>2</sub> genes revealed that ammodytoxin C and ammodytin L genes contain in the 4th intron nearly the same 630 bp retroposon sequence. The sequence is 75 % identical to *Artiodactyla* consensus ART-2 retroposon. It is widely dispersed in the genome of *Vipera ammodytes* as we confirmed by Southern blot analysis. The nucleotide sequences of ART-2 retroposon in both PLA<sub>2</sub> genes differ for only 2.4 %. The detection of an ART-2 retroposon in *Vipera ammodytes* genome in addition to genomes of family *Bovidae* disagree with the present understanding of SINEs distribution (D Kordiš and F. Gubenšek, *Nature Genetics* 1995, in press). To examine a possible presence of the ART-2 retroposon in other vertebrate classes, we performed a Southern blot analysis using genomic DNA from the members of four vertebrate classes *Mammalia*, *Aves*, *Reptilia*, *Amphibia* and *Arthropoda* (tick *Ixodes ricinus*). This experiment has shown that in tested *Mammalia* ART-2 retroposon is not present outside the family *Bovidae*, neither is it present in chicken, lizard, frog and tick genomes. It is, however, present beside *Vipera ammodytes* in the genomes of all three tested members of *Viperinae* (*Vipera palestinae*, *Echis coloratus*) and *Crotalinae* (*Bothrops alternatus*) subfamilies. These results may indicate that the amplification of ART-2 retroposon in snakes occurred before the divergence of *Viperinae* and *Crotalinae* subfamilies. The horizontal transfer thus seems to be the only possible explanation of the origin of this retroposon in *Vipera ammodytes* and other viperid snakes.

EFFECTS OF CROTOXIN ON MURINE OMOHYOID MUSCLE:  
SPECTROFLUORIMETRY, FLUORESCENCE IMAGING, AND  
CREATINE KINASE RELEASE

<sup>1,3</sup>Paulo A. Melo, <sup>2</sup>Craig F. Burns, <sup>1</sup>Charlotte L. Ownby, and <sup>2</sup>James T. Blankemeyer

<sup>1</sup>Department of Physiological Sciences and <sup>2</sup>Zoology, Oklahoma State University, Stillwater, OK, USA and <sup>3</sup>Departamento de Farmacologia Basica e Clinica, ICB, UFRJ, Rio de Janeiro, RJ, Brasil

To study the myotoxicity of crotoxin (CTX), we used an electrochromic fluorescent dye, DI-4-ANEPPS (Di-4), to measure the changes in membrane potential of murine omohyoid muscle. Before CTX (10 µg/ml) was added to the medium, the muscle was incubated for 30 min in physiologic saline (PSS) containing 1 µM of Di-4. The preparation was illuminated using a dual-wavelength spectrofluorimeter (PTI), and the Di-4 fluorescence was recorded at 1 min intervals or continuously for 10-20 min. CTX caused a significant depolarization, relative to the negative controls within 60 sec of exposure. Depolarization by valinomycin (1 µM in a 100 mM KCl-enriched PSS) served as a positive control. The same preparation was examined with a fluorescence imaging system. Within 3 min of CTX exposure, fluorescence images revealed muscle contractions, bright spots along the fibers and differing sensitivity to CTX between muscle bundles. The quantitative fluorescence data agreed with the PTI results. We also measured the increase of creatine kinase (CK) release above the basal level. CTX (10 µg/ml) caused an increase of CK release from a basal level of  $0.36 \pm 0.10$  U/g/hr (N=6) to  $4.82 \pm 1.70$  U/g/hr (N=6) after 10 min.

Paulo A. Melo has a CNPq Posdoctoral Fellowship.

## THE EFFECT OF ANTIBODIES DIRECTED AGAINST THE CROTOXIN ON THE FROG NERVE - MUSCLE PREPARATION

<sup>1</sup>I. Horvat-Žnidaršič, <sup>2</sup>V. Choumet, <sup>2</sup>C. Bon, <sup>1</sup>D. Šuput

<sup>1</sup>Institute of Pathophysiology, School of Medicine, P.O. Box 11, Ljubljana

<sup>2</sup>Unite des Venins, Institut Pasteur, 25. rue du Dr ROUX, Paris Cedex 15, France

Crotoxin is the major neurotoxic component of the venom of the South American rattlesnake, *Crotalus durissus terrificus*, which blocks neuromuscular transmission at a presynaptic level. The crotoxin molecule is composed of a basic phospholipase A<sub>2</sub>, called component-B (CB), and an acidic, nonenzymatic and nontoxic subunit called component-A (CA). CA enhances the pharmacological efficiency of the CB subunit by restricting its binding to specific target sites.

Anti-CA and anti-CB monoclonal antibodies (MAb) were shown to recognize three epitopic regions on CA and four on CB. One epitopic region on CA and four on CB are involved in the neutralization of the lethal potency of crotoxin (1). It was thus interesting to test wheather the neutralization of crotoxin's lethal potency *in vivo* could be correlated with a direct effect on its toxicity on isolated neuromuscular junctions.

The MAbs directed against the crotoxin were tested on the frog neuromuscular junctions. Muscles were stimulated directly and indirectly. Twitch responses were registered before and after the addition of the toxin or the mixture toxin:MAb. The effects of neutralizing MAbs were compared with those of some non neutralizing MAbs. Non immune Abs were used as a control.

In general, a good agreement was observed between the *in vivo* neutralization potency of Mabs and their ability to prevent the *in vitro* blocking action on frog neuromuscular transmission: neutralizing anti-CB Mabs completely prevented the crotoxin blocking effect *in vitro* and non neutralizing anti-CA Mabs were also inactive on the nerve-muscle preparation. It remains however to be clarified why some of the non neutralizing anti-CB Mabs, which interact with the epitope region B-1a partially block the effect of crotoxin on the frog nerve-muscle preparation.

(1) Choumet, V. *et al.* (1992) Molecular Immunology, 29(7/8), 871-882.



## BETA TAIPOXIN AS A NOVEL MITOGEN HAVING REGENERATIVE WOUND HEALING PROPERTY

Binie Ver Lipps, Ophidia Products, Houston, Texas

A purified mitogenic factor has been isolated from the venom of *Oxyuranus scutellatus* by high pressure liquid chromatography (HPLC). The fractionation of the venom resolved nine major fractions. Initially each fraction was tested on pheochromocytoma (PC12) cells for mitogenic activity and fractions 6 and 7 showed the highest activity. The potent mitogen in fractions 6 and 7 were concentrated and refractionated by HPLC under identical conditions to obtain 100% pure protein referred to as Oxynor. The molecular weight of Oxynor is 13,500 daltons; it is stable and non toxic to mouse.

On sequencing the purified fractions 6 and 7 were identified as betataipoxin-1 and betataipoxin-2, respectively. The intact molecule of taipoxin has molecular weight of 45,600 daltons, is very toxic and consists of three immunologically distinct chains: alpha, beta and gamma. The regenerative mitogenic activity is associated with the beta chain which is Oxynor.

Incorporation of 0.1  $\mu\text{g}/\text{ml}$  of Oxynor into Dulbecco's modified Eagle's medium (DMEM) promotes growth of various types of cells of primary and established cell lines equivalent to DMEM containing 10% serum. In vivo, experimentally cut portions of mouse skin treated with a topical application of Oxynor healed faster than the controls. In humans Oxynor heals chronic wounds without scarring. Thus, (1) Oxynor can replace serum supplements for cell culture and (2) Oxynor has application to wound healing and burn treatment because of its neuroregenerative and keratinocyte activities.

Biochemical and neurotoxicological characterization of paradoxin, a  $\beta$ -neurotoxin from Australian Inland Taipan snake (*Oxyuranus microlepidotus*) venom.

F. DORANDEU, I. PERNOT-MARINO, G. LALLEMENT (Centre de Recherches du Service de Santé des Armées, Unité de Neurotoxicologie, 24 Av. des Maquis du Grésivaudan, BP87, 38702 La Tronche Cédex, France).

Paradoxin, purified from the Australian Elapid snake *Oxyuranus microlepidotus* venom is a little-studied toxin that belongs to the  $\beta$ -neurotoxin family, a group of potent snake neurotoxins having PLA<sub>2</sub> (phospholipase A<sub>2</sub>) catalytic properties. Paradoxin (PDX) was first described 15 years ago as a taipoxin-like toxin (taipoxin is a three chain  $\beta$ -neurotoxin isolated from the Taipan snake, *O.s.scutellatus*). Since 1979, very few studies have been done on PDX and that led us to investigate its neurotoxic effects, with a particular focus on its potential convulsive activity, as some  $\beta$ -neurotoxins have been observed to induce seizures. The main features of toxicity after peripheral injection (ip) of PDX in rats were similar to the ones described for other  $\beta$ -neurotoxins. After intracerebroventricular (icv) injection of different doses through a stereotaxically implanted cannula, a four step neurotoxic syndrome developed. No seizures were observed by EEG recording. The mean lethal dose by icv route was ca. 1.2  $\mu$ g/kg. Histological examination of the brain of the rats killed by PDX did not show major target areas. *In vitro* experiments using rat hippocampal and striatal minislices were then carried out in order to determine the major neurochemical disturbances induced by PDX. In superfusion experiments, PDX appeared to be able to induce, by itself, a slight increase of ACh and Glu release from hippocampal preparations. Our preliminary results on release of DA from striatal minislices showed that PDX significantly increased the K<sup>+</sup> evoked release after 6 or 16 min of contact with the toxin during the course of the superfusion. However preincubation with PDX for a longer period before the superfusion began led to an apparent suppression of ACh, Glu or DA evoked release. This could have been caused by an impairment of the uptake mechanism and/or an alteration of the membranes. Some of these effects have been described for other  $\beta$ -neurotoxins. However, these *in vitro* results are not sufficient to explain the complex neurotoxic syndrome observed that might involve several types of neurotransmission.

# INDUCTION OF GIANT MINIATURE END-PLATE POTENTIALS DURING NEUROMUSCULAR BLOCKADE BY THE PRESYNAPTIC SNAKE NEUROTOXIN TEXTILOTOXIN

G.M.Nicholson and H.I.Wilson

Department of Health Sciences, University of Technology, Sydney, NSW 2007, Australia

Recent purification and subsequent studies on the structure of textilotoxin, isolated from the venom of the Australian common brown snake *Pseudonaja textilis*, have revealed that it is the largest and structurally most complex of all known snake neurotoxins. It is comprised of five subunits arranged in the form ABCD<sub>2</sub> giving a total  $M_r$  of 70,551 with an LD<sub>50</sub> of 0.6 µg/kg i.v. in mice. The present study investigated the actions of purified textilotoxin, on neuromuscular transmission in isolated nerve-muscle preparations from the cane toad *Bufo marinus*. Initial twitch tension experiments in sciatic-sartorius nerve-muscle preparations revealed a triphasic pattern of changes in muscle tension and a irreversible binding action of textilotoxin (10 µg/ml) similar to other snake β-neurotoxins. This was characterised by an initial depression of twitch tension, followed by a period of enhanced contractility, eventually leading to a reduction in tension to complete neuromuscular blockade. These actions on muscle tension were investigated further by assessing the action of textilotoxin on end-plate potential amplitude (e.p.p.) in sciatic-iliofibularis nerve-muscle preparations. Muscle contractions were prevented by reducing the quantal content of evoked e.p.ps to below threshold by raising the external magnesium concentration to 9-11 mM. These experiments revealed a similar triphasic alteration of the nerve-evoked release of acetylcholine from the motor nerve terminal. These actions on acetylcholine release were confirmed to be of a presynaptic origin since the modal amplitude of miniature end-plate potentials (m.e.p.ps) was not reduced and in twitch tension experiments the muscle still contracted in response to direct muscle stimulation when nerve-evoked release was completely blocked. Interestingly dramatic effects were observed on the spontaneous release of acetylcholine, including an marked increase in m.e.p.p. frequency, a skewing of the m.e.p.p. amplitude frequency histogram to the right, and a resultant increase in the number of 'giant' m.e.p.ps defined as being greater than 2.5 times the control mean m.e.p.p. amplitude. These results indicate that textilotoxin causes a presynaptic blockade of neuromuscular transmission involving a disruption of the regulatory mechanism that controls acetylcholine release.

## Localization and expression of *Trimeresurus flavoviridis* phospholipases A<sub>2</sub> in venom gland

<sup>1</sup>Tomohisa Ogawa, <sup>2</sup>Hitoshi Onoue, <sup>3</sup>Kazunori Nakagawa, <sup>2</sup>Shintarou Nomura, <sup>3</sup>Katsuo Sueishi, <sup>4</sup>Shosaku Hattori, <sup>5</sup>Hiroshi Kihara, and <sup>1</sup>Motonori Ohno

<sup>1</sup>Lab. of Biochemistry, Dept. of Chemistry, Fac. of Science, Kyushu University, Fukuoka 812-81, <sup>2</sup>Dept. of Pathology, Osaka University Medical School, Suita 565, <sup>3</sup>Lab. of Pathology I, Fac. of Medicine, Kyushu University, Fukuoka 812-82, <sup>4</sup>Institute of Medical Science, University of Tokyo, Kagoshima 894-15, <sup>5</sup>Biotech. Res. Lab., Takara Shuzo Co. Ltd, Shiga 520-21, Japan

Snake venoms contain many phospholipase A<sub>2</sub> (PLA<sub>2</sub>) isozymes in large quantity. The localization and the mechanism of expression and regulation of PLA<sub>2</sub> isozymes in the venom gland are of great interest because none has been known. The venom gland is resistant to venom proteins in spite of their strong cytotoxicity. The mechanism to prevent cell damage from its own venom proteins is also of interest. In this study, the localization and expression profiles of phospholipases A<sub>2</sub> (PLA<sub>2</sub>s) in *Trimeresurus flavoviridis* venom gland were studied by means of *in situ* hybridization and immunohistochemical techniques. Venom gland cells are tightly arrayed in a single layer along the inlet-like ducts in which venom proteins are stored. mRNAs for PLA<sub>2</sub>s were detected at the high level in cytoplasm. Using the immunohistochemical technique with polyclonal anti-Asp-49-PLA<sub>2</sub> antibody, Asp-49-PLA<sub>2</sub> and possibly its isozymes were detected in intracellular granules and in venom ducts. The intracellular granules containing PLA<sub>2</sub> proteins appear to be transferred from the nucleus toward the outer membrane facing the duct and then be secreted.

## THE NEPHROTOXIC ACTION OF THE CRUDE *Bothrops insularis* VENOM AND OF PLA<sub>2</sub>- CONTAINING A FRACTION.

<sup>1</sup>D'Abreu, A.C.F., <sup>2</sup>Rodrigues-Simioni, L., Cogo, J.C. and <sup>1</sup>Cruz-Höfling, M.A.

<sup>1</sup>Dept. of Histology and Embriology, Institute of Biology, UNICAMP, and <sup>2</sup>Dept. of Pharmacology, Faculty of Medical Sciences, UNICAMP, Campinas, SP, Brazil.

*Bothrops insularis* (*jararaca-ilhoa*) is restricted to the Island Queimada Grande off the coast of the State of São Paulo. This species differs from other Brazilian *Bothrops* in that it feeds almost exclusively on birds. In mammalian and avian muscle, the venom causes myonecrosis, vascular disturbances and local hemorrhage. The present work investigates the morphological and ultrastructural alterations induced by *B. insularis* venom and a fraction with phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity in the kidneys of 5-12 day -old chicks. Twenty and 80 µg of ml and 10 and 40µg of PLA<sub>2</sub>/ml were injected (0.1 ml) into the left pectoral muscle. The morphological changes were examined 3 and 24h later. The effects caused by the venom were are more extensive than those caused by PLA<sub>2</sub> fraction. However, the degenerative changes caused by the latter in the renal corpuscle and the expressive tubular necrosis more marked than those induced by the venom. The convoluted proximal tubules were the most affected and the changes included widening of the intercellular spaces (with eventual cell detachment), vacuolation, and a irregular profile of the brush border. There was also a higher development of the endocytotic apparatus the vacuoles of which had heterogeneous content. The renal corpuscles were frequently congested and the podocytes presented enlarged cisternae of the rough endosplasmic reticulum. In the cases of acute degeneration (24 h), the filtering barrier could be completely ruptured. Variable degrees of pathological changes were observed among the different segments of the nephron in the same animal. The cortical parenchyma was more affected than the medullar. We conclude that both the venom and the PLA<sub>2</sub> fraction of *B. insularis* have a nephrotic effect the molecular mechanism of wich has yet to be defined.

Support: SAE, FAPESP, CNPq, FAEP

## NEUROMUSCULAR EFFECTS OF A FRACTION WITH PHOSPHOLIPASE A<sub>2</sub> ACTIVITY ISOLATED FROM *Bothrops insularis* VENOM.

Cogo, J.C.<sup>1</sup>; Prado-Franceschi, J.<sup>2</sup>; Pinto-Corrado, A.<sup>3</sup>; Giglio, J.R.<sup>4</sup>; Donato, J.L.<sup>2</sup>; Rodrigues-Simioni, L.<sup>2</sup>

<sup>1</sup>Dept. of Physiology and Biophysics, I.B., UNICAMP, Campinas, SP, Brazil; <sup>2</sup>Dept. Pharmacology, F.C.M., UNICAMP, and Departments of <sup>3</sup>Pharmacology and <sup>4</sup>Biochemistry, FM-USP, Ribeirão Preto, SP, Brazil.

The *in vitro* effects of a fraction with phospholipase A<sub>2</sub> activity isolated from the venom of *Bothrops insularis* were studied. The crude venom was fractionated by gel filtration on Sephadex G-150. The peak with PLA<sub>2</sub> activity was further purified by ion exchange chromatography on a DEAE-Sephadex column. The resulting peak with PLA<sub>2</sub> activity was tested in neuromuscular preparations (phrenic nerve-diaphragm and chick *biventer cervicis* muscle preparations). In mammalian muscle, the PLA<sub>2</sub>-containing fraction (20 and 40  $\mu\text{g/ml}$ ) induced an initial increase in the twitch tension, an increase in the frequency of miniature end plate potentials, and a dose-dependent, irreversible blockade. The replacement of 1.8 mM Ca<sup>2+</sup> with 4 mM Sr<sup>2+</sup> inhibited the neuromuscular blocking effect of the fraction. In the avian preparation, this fraction induced blockade at doses up to 40  $\mu\text{g/ml}$  but did not affect the responses to ACh and K<sup>+</sup>. The blockade could be antagonized by lowering the incubation bath temperature from 37°C to (22 - 25 °C). Contracture was observed only in the avian preparation. These results suggest that the blocking effect of the PLA<sub>2</sub>-containing fraction is due to an action at the neuromuscular junction, mainly at pre-synaptic sites. This fraction exhibits most of the pharmacological effects of the crude venom (COGO et al, *Toxicon* 31: 1237-47, 1993).

Supported: FAPESP; FAEP/UNICAMP.

## HEPATIC MORPHOLOGICAL ALTERATIONS INDUCED BY *BOTHROPS INSULARIS* VENOM AND A FRACTION WITH PLA<sub>2</sub> ACTIVITY IN CHICKS.

<sup>1</sup>Paronetto, C.C., <sup>2</sup>Cogo, J.C., <sup>2</sup>Rodrigues-Simioni, L. and <sup>1</sup>Cruz-Höfling, M.A.

<sup>1</sup>Dept. of Histology and Embriology, Institute of Biology, UNICAMP, and <sup>2</sup>Dept. of Pharmacology, Faculty of Medical Sciences, UNICAMP, Campinas, SP, Brazil.

Local necrosis is a frequent consequence of envenomation by viperids and crotalids and may lead to amputation of the affected member. This is particularly true for bites by snakes of the genus *Bothrops*. The range of pathological effects include systemic disturbances evoked by coagulant, fibrinolytic and hemolytic enzymes of the venom. The liver constitutes an important organ involved in such systemic reactions since it produces thrombin and fibrinogen, both of which are essential for normal hemostasis. The present work investigates the effects of *Bothrops insularis* venom (V) and a fraction with phospholipase (PLA<sub>2</sub>) activity. Twenty and 80 µg of v/ml and 10 and 40 µg, of the fraction/ml were injected i.m. (0.1 ml into the pectoral muscle of 5-12 day-old chicks (Hy-line-Lineage). Three and 24h later the birds were anesthetized with ether and sacrificed by the intra-cardiac perfusion of fixative (buffered 10% formalin or Kornovsky fixative). The pathological changes induced by V and the PLA<sub>2</sub> fraction were both time and dose-dependent with V producing more intense reactions. The loss of the hepatic architecture made visualization of the sinusoids, central lobular vein and the portal spaces difficult. At high doses, vacuolar hepatocyte degeneration, mitochondrial cristae disruption and the appearance of very dark hepatocytes were common findings. There was a striking decrease in the amount of glycogen deposits as compared to the controls. Non-parenchymatous cells, such as pitt cells and Kuppfer cells appeared activated and outnumbered those seen in the controls. The PLA<sub>2</sub> fraction had a stronger hemorrhagic effect in the hepatic tissue. We conclude that *B. insularis* venom has a systemic action with a probable involvement of the hepatic system.

Supported: FAEP, SAE, FAPESP, CNPq.

## SPECIFICITIES OF Asp49, Asp35 AND Lys49 PHOSPHOLIPASE A<sub>2</sub> ENZYMES ON SKELETAL MUSCLE LIPID SUBSTRATES

<sup>1</sup>J.E. Fletcher, <sup>1</sup>M-S. Jiang, <sup>2,3</sup>H.S. Selistre de Araujo and <sup>2</sup>C.L. Ownby

<sup>1</sup>Medical College of PA and Hahnemann Univ., Philadelphia, PA U.S.A.

<sup>2</sup>Oklahoma State University, Stillwater, OK U.S.A.

<sup>3</sup>Universidade Federal de São Carlos, São Carlos SP, Brazil

The overall enzymatic activities and substrate specificities of two Lys49 phospholipase A<sub>2</sub> (PLA<sub>2</sub>) enzymes, bothropstoxin (BThTx-I) from *Bothrops jararacussu* venom and ACL myotoxin (ACLMT) from *Agkistrodon contortrix laticinctus* venom, were compared with three Asp49 PLA<sub>2</sub> enzymes from *Naja nigricollis*, *Naja naja atra* and *Naja naja kaouthia* venoms and an Asp35 PLA<sub>2</sub> from *Apis mellifera*, using radiolabeled primary cultures of human skeletal muscle. The relative overall order of enzymatic activity was *A. mellifera*  $\approx$  *N.n. atra*  $\approx$  *N.n. kaouthia*  $\approx$  *N. nigricollis*  $\gg$  BThTx-I  $\approx$  ACLMT. While the values for hydrolysis of phosphatidylethanolamine (PE), phosphatidycholine (PC), phosphatidylserine and phosphatidylinositol were all determined, the major differences involved PE and PC. The *N.n. atra* and *N.n. kaouthia* PLA<sub>2</sub> enzymes at 10 nM (n = 3 for each) had substrate preferences of PE (61  $\pm$  3% and 88  $\pm$  2%, respectively; % hydrolysis, mean  $\pm$  SD) over PC (4  $\pm$  2% and 22  $\pm$  3%). In contrast, the *Naja nigricollis* PLA<sub>2</sub> at 100 nM (n = 3) had a slight preference of PC (56  $\pm$  4%) over PE (47  $\pm$  4%). *A. mellifera* PLA<sub>2</sub> at 10 nM (n = 3) and BThTx-I (n = 12) and ACLMT (n = 10) each at 5  $\mu$ M had no preferences for PC (41  $\pm$  4%, 71  $\pm$  15%, 22  $\pm$  15%, respectively) or PE (55  $\pm$  14%, 71  $\pm$  12%, 43  $\pm$  24%). The catalytic activity of Lys49 PLA<sub>2</sub> enzymes is low relative to Asp49 and Asp35 PLA<sub>2</sub> enzymes. The substrate preference of the Lys49 enzymes is most like the structurally unusual *A. mellifera* PLA<sub>2</sub>, which contains an Asp35 functionally identical to Asp49 of other PLA<sub>2</sub>s (Science 250 1563, 1990). [H.S.S.A. has a fellowship from CNPq, Brazil.]



## INHIBITION OF SYNAPTOSOMAL BINDING OF $^{125}\text{I}$ - DABOIATOXIN BY PYTHON SERUM

<sup>1</sup>M.M. Thwin, <sup>1</sup>P. Gopalakrishnakone, <sup>2</sup>R. Yuen and <sup>2</sup>C.H. Tan, Venom and Toxin Research Group, Departments of <sup>1</sup>Anatomy and <sup>2</sup>Biochemistry, Faculty of Medicine, National University of Singapore, Republic of Singapore

Daboiatoxin (DbTx), a phospholipase A<sub>2</sub> neurotoxin purified from *Daboia russelii siamensis* venom and radioiodinated to high specific activity (500-700 Ci/mmol), bound specifically with high affinity to synaptosomes from Wistar rat, snake (*Python reticulatus*), and little civet cat (*Viverricula indica*). While rat brain synaptosomes contained two different binding sites of high and low affinities ( $K_{d1} = 5-8$  nM;  $B_{max1} = 6$  pmoles/mg and  $K_{d2} = 80$  nM;  $B_{max2} = 20$  pmoles/mg), synaptosomes from python ( $K_d = 6.2$  nM;  $B_{max} = 26$  pmoles/mg) and civet cat ( $K_d = 5.8$  nM;  $B_{max} = 22$  pmoles/mg) exhibited only a single class of high affinity binding sites. Binding of  $^{125}\text{I}$ -DbTx to rat synaptosomes was displaceable by  $1\mu\text{M}$  and above concentrations of crotoxin, mojave toxin, ammodytoxin A and *Crotalus atrox* PLA<sub>2</sub>, but  $^{125}\text{I}$ -DbTx bound to python synaptosomes was indisplaceable by those neurotoxins, indicating relatively tighter binding of  $^{125}\text{I}$ -DbTx by the latter. Competition binding experiments with a range of neurotransmitter antagonists, and potent K<sup>+</sup> and Ca<sup>++</sup> channels blockers suggest that the receptor target for DbTx is neither adrenergic, nicotinic and muscarinic cholinergic, GABA, opiate, dopamine, and serotonin receptors, nor any of the subtypes of K<sup>+</sup> and Ca<sup>++</sup> channels. DbTx strongly inhibited the uptake of <sup>3</sup>H-choline whereas <sup>3</sup>H-GABA uptake was not affected. Compared to sera of normal human (control) or civet cat, python serum was equally effective as polyclonal rabbit anti-DbTx antisera in inhibiting  $^{125}\text{I}$ -DbTx binding ( $\text{IC}_{50}$  of both sera = 1:6,000 dilutions) to rat synaptosomes, or to python and civet cat synaptosomes. This finding is indicative of the presence of  $^{125}\text{I}$ -DbTx binding inhibitor in the python serum. Further work in defining this factor is in progress.

## THE SCREENING OF *Bothrops* VENOMS FOR NEUROTOXIC ACTIVITY USING THE CHICK *BIVENTER CERVICIS* PREPARATION.

Zamuner, S.R., Prado-Franceschi, J., Rodrigues-Simioni, L.

Department of Pharmacology, Faculty of Medical Sciences, PO Box 6111, UNICAMP, 13084-100, Campinas, SP, Brazil.

Since the early 1980's, the venoms of several species of *Bothrops* including *B. jararacussu*, *B. insularis* and *B. lanceolatus* have been shown to possess a neurotoxic action. The chick *biventer cervicis* preparation provides a convenient means of screening for such activity (Harvey *et al.*, *Toxicon* **32**, 257, 1994). We have used this preparation to examine the distribution of neurotoxicity in the venoms of several Brazilian species of *Bothrops* (*B. jararaca*, *B. jararacussu*, *B. moojeni*, *B. neuwiedi* and *B. erythromelas*). As a positive control, we used the venom of the South American rattlesnake *Crotalus durissus terrificus*. The venoms of *B. jararaca*, *B. jararacussu*, *B. moojeni* and *B. erythromelas* (100-200 µg/ml, n=3 each) induced dose-dependent muscle contracture and twitch-tension blockade as well as inhibition of the responses to ACh and K<sup>+</sup>. The venom of *B. neuwiedi* was 4-10 fold more potent than the other venoms (active range: 10-50 µg/ml, n=3) and was similar in action to that of *C. d. terrificus* in that it blocked twitch-tension responses without affecting the action of ACh or K<sup>+</sup>. In addition, this venom did not induce muscle contracture. *In vivo*, *B. neuwiedi* venom caused head-drop, loss of balance and respiratory failure, symptoms which were not observed with the other venoms. The latter caused predominantly hemorrhagic effects. These observations for *B. neuwiedi* are therefore similar to those previously reported for *B. insularis* (Cogo *et al.*, *Toxicon* **31**, 1237, 1993). The i.m. LD<sub>50</sub> (mg/kg with 95% confidence limits) was 5.0 (3.6-6.8) for *B. jararaca*, 2.7 (2.1-2.8) for *B. moojeni*, 0.67 (0.2-2) for *B. neuwiedi*, 0.55 (0.3-0.8) for *B. erythromelas*, 0.038 (0.033-0.043) for *C. d. terrificus*, and >300 for *B. jararacussu*. These results indicate a lack of correlation between the *in vitro* effects and the LD<sub>50</sub> values. Using the chick *biventer cervicis* preparation the venoms could be divided into two groups: those with a potent neurotoxic action (*B. neuwiedi* and *B. insularis*) and those with weak neuromuscular blocking activity (*B. jararaca*, *B. jararacussu*, *B. moojeni* and *B. erythromelas*).

Financial Support: CAPES and FAEP/UNICAMP

## NATURE OF THE POSTSYNAPTIC ACTION OF CROTOXIN AT THE GUINEA-PIG END-PLATE

O. Vital Brazil and M.D. Fontana

Department of Pharmacology, Faculty of Medical Sciences, UNICAMP,  
Campinas, P.O. Box 6111, SP, Brazil

Crotoxin induces desensitization of the nicotinic receptor of the Torpedo marmorata electric organ (Bon et al., 1979). The aim of our study was to elucidate if crotoxin postsynaptic effect on mammalian end-plates is also due to receptor desensitization. Investigation of the effect of 4-aminopyridine (4-AP) on the blockade of the m.e.p.ps and carbachol-induced depolarization in the guinea-pig diafragma preparation was employed for that purpose since the aminopyridine inhibits end-plate receptor desensitization (Vital Brazil et al., 1983, 1989), and the guinea-pig diaphragm in contrast to mouse and rat diaphragms is very sensitive to the postsynaptic action of crotoxin. The experiments were carried out on horizontally mounted hemidiaphragms in an organ bath with Tyrode solution at 37°C oxygenated with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. The potentials were measured with conventional techniques using glass microelectrodes filled with 3 M KCl. Crotoxin even in small concentrations (2 ug/ml) reduced the amplitude of the m.e.p.ps before blocking them. The blockade was antagonized by 4-AP but not by neostigmine. On the other hand, 4-AP did not induce the reappearance of the m.e.p.ps blocked by dTc. 4-AP also antagonized the inhibition produced by crotoxin on the carbachol-induced depolarization of the guinea-pig end-plates. Receptor desensitization is suggested by these results to be also the cause of the postsynaptic effect on the guinea-pig end-plate.

## ELECTROPHYSIOLOGICAL AND ULTRASTRUCTURAL EFFECTS OF *Micrurus nigrocinctus* VENOM ON THE NEUROMUSCULAR JUNCTION.

Goularte, F.C.<sup>1</sup>, Cruz-Höfling, M.A.<sup>2</sup>, Rodrigues-Simioni, L.<sup>3</sup>

<sup>1</sup>Dept. Pharmacological Sciences, CCBPS, UNIMEP, Piracicaba, SP, Brazil and Departments of <sup>2</sup>Histology and Embriology and <sup>3</sup>Pharmacology, UNICAMP, Campinas, SP, Brazil

*Micrurus nigrocinctus* is the most abundant coral snake in Central America (BOLANOS, Revta Costa Rica Cienc. Méd. 3: 165, 1982). The venom of this species induced a dose-dependent (1-20 µg/ml) depolarization in the isolated mouse phrenic nerve-diaphragm preparation. D-Tubocurarine (dTc, 10 µg/ml) and α-bungarotoxin (α-BuTX, 3-5 µg/ml) were able to partially protect against the depolarization induced by the venom (10 µg/ml), suggesting the involvement of postsynaptic cholinergic receptors at least in the initial stages of envenomation. *M. nigrocinctus* venom (10 µg/ml) increased the frequency and amplitude of miniature and-plate potentials (mepps) during the first 10-20 min of incubation. Subsequently, the mepps progressively decrease and disappeared after 60 min. These responses were accompanied by ultrastructural changes involving the nerve terminals, the postsynaptic junctional folds and the muscle mitochondria. The synaptic gutter was shallow and very often "shrunk" terminals with omega-shaped axolemmal indentations and a decreased number of synaptic vesicles were present. A common finding was the presence of numerous finger-like membrane-bounded bodies interposed between the terminal and the Schwann cell or the postsynaptic sarcolemma. Incubating the preparations at room temperature (24 - 26°C) or pre-incubated the venom with specific antivenom reduced the number and intensity of the ultrastructural alterations. These observations suggest the possible involvement of PLA<sub>2</sub> (ARROYO *et al.*, Comp. Biochem. Physiol. 87B(4): 947, 1987, GUTIERREZ *et al.*, Brazilian J. Med. Biol. Res. 24: 701, 1991). There were a good correlation between the electrophysiological and morphological effects of the crude venom. We conclude that *M. nigrocinctus* venom has a presynaptic action in the initial stages of intoxication and that this is followed by a postsynaptic action which is the most important in causing neuromuscular blockade. The direct action of the venom on the muscles may also contribute to the irreversible blockade

Financial Support: FAPESP, CNPq, FAEP/UNICAMP.

MODE OF ACTION OF DUVERNOY'S GLAND EXTRACTS FROM THE  
COLUBRIDAE *DRYADOPHIS BIFOSSATUS* IN THE CHICK BIVENTER  
CERVICIS NERVE-MUSCLE PREPARATION

M.D. Fontana, M.G. Heleno and O. Vital Brazil  
Departament of Pharmacology, Faculty of Medical Sciences, UNICAMP,  
Campinas, P.O. Box 6111, SP, Brazil

Extracts and secretion of the Duvernoy's gland, a cephalic gland of the Colubridae are venomous, inducing, in several cases motor and respiratory paralysis in experimental animals. Kraus (1922, 1924) and Brazil & Vellard (1926) showed that extracts of the Duvernoy's gland plus the supralabial gland (which is associated with the Duvernoy's gland) from the aglyphous Colubridae *D. bifossatus* are very toxic, eliciting flaccid paralysis in pigeons, rabbits and Hylae. Local effects, blood coagulation and indirect haemolysis were not produced. The present study is part of a pharmacological one of the Duvernoy's gland extracts and secretion from several Brazilian Colubridae. It was carried out in the biventer cervicis muscle prepared according to Ginsborg and Warriner (1960) Krebs solution at 37°C, oxygenated with 95% O<sub>2</sub>, 5% CO<sub>2</sub> was the physiological solution used. The muscle was indirectly stimulated with supramaximal pulses of 0.2 ms, 0.05 Hz and also by Ach addition to the bath. Direct muscle stimulation was carried out in curarized (dTc 5 µg/ml) preparations stimulated with 80 V, 0.2 ms, 0.05 Hz pulses. The muscle contractions were isometrically recorded in a Gould RS 3400 Physiograph. The extracts of the Duvernoy's gland from *D. bifossatus* induced an irreversible blockade in the biventer cervicis nerve-muscle preparation and inhibited the contracture of the muscle produced by Ach. Twitch depression in curarized preparation was not induced. These results suggest that the active component of the extracts is a neuromuscular blocking toxin acting at the postsynaptic membrane of the neuromuscular junction. Similar mechanism of neuromuscular blocking action is produced according to Levinson and coworkers (1976) by the secretion of the Duvernoy's gland from the African Colubridae *Boiga blandingi*.

PHARMACOKINETICS OF  $^{125}\text{I}$ -LABELLED *WALTERINNESIA*  
*AEGYPTIA* VENOM: FLASH DISTRIBUTION OF THE VENOM AND  
ITS TOXIN

M. Ismail, M.A. Abd-Elsalam and M.S. Al-Ahaidib

The Antivenom and Vaccine Production Center, King Fahad National Guard  
Hospital, Riyadh 11426, Saudi Arabia

Fractionation of *W. aegyptia* venom was carried out using Amberlite CG-50 and  $(\text{NH}_4)\text{HCO}_3$  elution gradient chromatography followed by gel filtration of the toxic fractions (FVII & FX) on Sephadex G-50. The most toxic fraction, FVII, yielded a single component that was lethal to mice in doses lower than  $2.5\text{ }\mu\text{g/kg}$  i.v. Following i.v. injection into rabbits, both the labelled venom and the labelled toxin mixed with non-labelled venom, followed a three compartment open pharmacokinetic model comprising a central compartment "Blood", a rapidly equilibrating "shallow" tissue compartment and a slowly equilibrating "deep" tissue compartment. The distribution of both the venom and toxin from the blood was very rapid. Mean distribution half-lives were 1.2 & 3.3 and 19.6 & 39.9 min for the venom and toxin for the shallow ( $t_{1/2\pi}$ ) and deep ( $t_{1/2\alpha}$ ) tissue compartments respectively. Mean overall elimination half-lives,  $t_{1/2\beta}$ , were 510 and 672 min for the venom and toxin respectively. Mean residence time in the body,  $\text{MRT}_b$ , and central compartment,  $\text{MRT}_c$ , was 897 & 196 and 862 & 97 min for the venom and toxin respectively indicating much greater affinity to the tissues. Most of the radioactivity was in the deep tissue compartment. At steady state the ratios of radioactivity in the deep tissue compartment: shallow tissue compartment: blood were 3.3:1.75:1 and 6.7:2.3:1 for the venom and toxin respectively. The results are in concordance with the rapid fatalities of victims bitten by the snake and the rapid death of experimental animal injected with the venom.

**ABSTRACT WITHDRAWN**

Modulation of cholinergic and glutamatergic neurotransmission in rat hippocampus by  $\alpha$ -dendrotoxin and the effect of the non-NMDA receptor antagonist DNQX. F. DORANDEU<sup>1</sup>, J. WETHERELL<sup>2</sup>, I. PERNOT-MARINO<sup>1</sup>, J.E.H. TATTERSALL<sup>2</sup>, G. LALLEMENT<sup>1</sup>, P. FOSBRAEY<sup>2</sup> (Centre de Recherches du Service de Santé des Armées, Unité de Neurotoxicologie, 24 Av. des Maquis du Grésivaudan, BP87, 38702 La Tronche Cédex, France; and <sup>2</sup>Chemical and Biological Defence Establishment, Porton Down, Salisbury, Wiltshire, SP4 OJQ, UK)

$\alpha$ -dendrotoxin ( $\alpha$ -DTX) is a 59 amino acid polypeptide isolated from the Eastern Green Mamba (*Dendroaspis angusticeps*) snake venom which blocks certain types of fast-activating, voltage-dependent potassium channels. Intracerebral injection of  $\alpha$ -DTX induces severe seizures and neuropathological changes in several areas of the brain as shown by benzodiazepine  $\omega$ 3 binding site measurements (LALLEMENT *et al.*, unpublished). In order to gain an insight into these effects of  $\alpha$ -DTX, we have studied the effect of the toxin on the release of the excitatory neurotransmitters acetylcholine (ACh) and glutamate (Glu) in the rat hippocampus. Microdialysis in area CA1 of freely moving rats failed to reveal any changes in extracellular levels of ACh and Glu after intracerebroventricular (icv) injection of  $\alpha$ -DTX (20 pmoles). *In vitro* experiments on superfused hippocampal minislices were then carried out to determine the effect of  $\alpha$ -DTX on basal release of radiolabelled or endogenous neurotransmitters.  $\alpha$ -DTX (1  $\mu$ M) increased the basal efflux of labelled ACh and Glu and of endogenous Glu and GABA. This increase in release was not prevented by the non-NMDA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 300 or 500  $\mu$ M). In contrast, extracellular recordings from hippocampal slices showed that the epileptiform activity induced by  $\alpha$ -DTX (250 nM) was blocked by DNQX (10  $\mu$ M), but not by the NMDA receptor antagonist D,L-2-amino-5-phosphonopentanoic acid (AP5, 100  $\mu$ M). We conclude that DNQX blocks  $\alpha$ -DTX-induced seizures by antagonism of postsynaptic non-NMDA receptors.



MORPHOLOGICAL CHARACTERISTICS OF MYOGLOBINURIC  
RENAL DAMAGE AFTER *PSEUDECHIS AUSTRALIS* ENVENOMATION

Ponraj D and Gopalakrishnakone P

Venom and Toxin Research Group, Dept of Anatomy, Faculty of medicine,  
National University of Singapore, Singapore 0511

The morphological changes in kidney after i.m injection of *Pseudechis australis* venom as well as a myotoxin (P A Myotoxin) causing myoglobinuria in mice were studied. LM and EM observations at various time intervals of envenomation showed acute myonecrosis of the injected calf muscle with features of acute tubular necrosis and cast nephropathy of the kidney. LM revealed evidence of a tubulopathy manifested by vacuolation, fragmentation and desquamation of the proximal tubular epithelial cells. Few tubules were dilated and lined by flattened cells. Necrotic tubular cellular debris within the lumen with areas of vascular congestion and haemorrhage were also seen. Areas of brush border blebbing of proximal tubules and numerous pigmented casts obstructing the whole lumen of the tubules were seen. Immunohistochemical staining for myoglobin using immunoperoxidase method revealed distinct staining of the tubular casts. EM studies showed features of acute tubulopathy with intra tubular casts composed of markedly electron dense material arranged in the form of round, globular structure obstructing the whole of the lumen were seen. The lysosomal system in the proximal tubules appeared to be activated with large and markedly atypical forms. Thus the generation of a "myoglobin cast nephropathy" is a major factor along with acute tubulopathy, in causing acute renal dysfunction following *P.australis* envenomation. However some areas with acute tubulopathy did not show significant numbers of tubular myoglobin casts. The possibility of direct tubular toxicity of *P.australis* venom also needs to be considered in the pathogenesis.

# ISOLATION AND CHARACTERIZATION OF A PLA<sub>2</sub> MYOTOXIN FROM PRAIRIE RATTLESNAKE (*CROTALUS VIRIDIS VIRIDIS*) VENOM

Charlotte L. Ownby and Terry R. Colberg

Department of Physiological Sciences, Oklahoma State University, Stillwater, OK 74078, USA

Myonecrosis induced by snake venoms can be due to different types of toxins including toxins which have the structure of a phospholipase A<sub>2</sub> (PLA<sub>2</sub>). We (Johnson and Ownby, 1993) previously isolated a myotoxin, ACL myotoxin, from the venom of the Broad-Banded Copperhead (*Agkistrodon contortrix laticinctus*) which has the PLA<sub>2</sub> structure, but lacks the enzymatic activity. Now, we have isolated a toxin, CVV myotoxin, from the venom of the Prairie Rattlesnake (*Crotalus viridis viridis*) which has high PLA<sub>2</sub> enzymatic activity and also causes myonecrosis in mice. The CVV myotoxin was isolated by cation exchange chromatography and has an estimated molecular weight of 14kDa, the same as the ACL myotoxin. A comparison of the N-terminal sequence (25 residues) with other PLA<sub>2</sub> proteins from snake venoms showed that the CVV myotoxin has high homology (76%) to agkistrotoxin from *Agkistrodon halys pallas* venom and to crotoxin B (72%) from *Crotalus durissus terrificus* venom, but considerably less homology (45%) to ACL myotoxin. CVV myotoxin induces the same type of myonecrosis typical of myotoxins from snake venoms having the PLA<sub>2</sub> structure, i.e., a rapid hypercontraction and clumping of myofibrils of affected skeletal muscle cells. When CVV myotoxin was incubated with *p*-bromophenacylbromide, the myotoxic activity was lost indicating that the enzymatic activity may be involved in myotoxicity. This myotoxin could be useful to study the structure-function relationship in the PLA<sub>2</sub> myotoxins.

Johnson, E.K. and Ownby, C.L. (1993) *Toxicon* 31, 243.

## DIFFERENT SENSITIVITY OF WHITE AND RED SKELETAL MUSCLES TO THE MYOTOXIC COMPONENTS OF SNAKE VENOMS

<sup>1,2</sup>Paulo A. Melo and <sup>1</sup>Charlotte L. Ownby

<sup>1</sup>Dept. of Physiological Sciences, Oklahoma State University., Stillwater, OK, USA and <sup>2</sup>Departamento de Farmacologia Basica e Clinica, ICB, UFRJ, Rio de Janeiro, RJ, Brasil

We examined the effect of crude snake venoms and isolated toxins on mouse skeletal muscles *in vitro*. Extensor digitorum longus (EDL) and soleus (SOL), white and red muscles respectively, were isolated as described by Melo and Suarez-Kurtz (1988, *Toxicon* 26, 87). Crude venoms from *Crotalus viridis viridis* (CVV), *Agkistrodon contortrix laticinctus* (ACL) and *Notechis scutatus scutatus* (NSS) were tested at a concentration of 25  $\mu\text{g/ml}$ . Increase of creatine kinase (CK) release (above basal levels) induced in each muscle by each venom or toxin was measured. EDL and SOL had the same range of basal CK release ( $0.30 \pm 0.06$  U/g/hr, N=26), weight (7-10 mg) and content of CK ( $3435.30 \pm 365.46$  U/g and  $3820.40 \pm 327.04$  U/g, N=8), but they had a different sensitivity to the myotoxic action of the tested venoms. The release of CK in EDL muscles was in the range of 24-60 U/g/hr after 60 min of exposure to each crude venom, whereas the increase of CK release in the SOL was in the range of 1.5-4.0 U/g/hr. Also crotoxin and myotoxin  $\alpha$  (10 and 25  $\mu\text{g/ml}$ , respectively) were more effective in EDL than in SOL muscles. Non-specific cytotoxic agents such as triton X-100 (0.01%) or polylysine (100  $\mu\text{g/ml}$ ) induced the same increase of CK release in both muscles.

Paulo A. Melo has a CNPq Postdoctoral Fellowship.

## EXPRESSION OF RECOMBINANT ACL MYOTOXIN IN *E. coli*

Heloisa S. Selistre de Araujo<sup>1,2</sup> and Charlotte L. Ownby<sup>1</sup>

<sup>1</sup>Department of Physiological Sciences, Oklahoma State University, Stillwater, Oklahoma 74078, USA, <sup>2</sup>Departamento de Ciências Fisiológicas, Universidade Federal de São Carlos, São Carlos, São Paulo 13565, Brasil

ACL myotoxin (ACLMT) is a 14 kDa Lys49 phospholipase A2 (PLA2) myotoxin devoid of detectable enzymatic activity. Although it is believed that PLA2 activity is not necessary for myotoxicity, the molecular mechanism of myotoxicity induced by Lys49 PLA2 myotoxins is still unclear. We have cloned and sequenced the ACL myotoxin cDNA from an *Agkistrodon contortrix laticinctus* venom gland cDNA library. To determine which amino acid residues in the toxin molecule are involved in myotoxic activity, we have developed an expression system for the mature ACL myotoxin for further site-directed mutagenesis studies. Based on the ACLMT cDNA sequence, we designed two primers for PCR amplification of the cDNA coding region of the mature protein. The forward primer had a *Bam* HI site and the reverse primer had an *Eco* RI site. PCR was developed using the Bluescript™ phagemid with the complete ACLMT cDNA insert as template. DNA sequence of a 400 bp PCR product showed that the amplified DNA corresponds to the mature protein coding region of the ACLMT cDNA and no mutations were introduced by the PCR. This PCR product was purified, double-restricted with *Bam* HI and *Eco* RI, and subcloned into a pET 28a™ vector. The new plasmid (pETMT) was used to transform BL21(DE3) competent *E. coli* cells. For expression, transformed cells were grown to log phase and then induced by the addition of 1 mM IPTG to the cell culture. The induction by IPTG led to the expression of a 20 kDa protein that strongly reacts with monoclonal antibody to purified ACL myotoxin.

H.S.S.A. has a fellowship from CNPq, Brazil

## ALTERED CALCIUM REGULATION IN RESPONSE TO SNAKE MYOTOXINS - CROTAMINE AND CARDIOTOXIN

M.D. Hubert, M-S. Jiang and J.E. Fletcher

Medical College of PA and Hahnemann Univ. Philadelphia, PA 19078  
USA

Crotamine is a myotoxic component of venom from the South American rattlesnake *Crotalus durissus terrificus*. This basic polypeptide consists of 42 amino acid residues and has a molecular weight of 4620. The mechanism of action is poorly understood, but may involve sarcolemmal  $\text{Na}^+$  channels and/or  $\text{Ca}^{2+}$  regulation by the sarcoplasmic reticulum (SR). Cobra venom cardiotoxins are also basic polypeptides that induce myotoxicity, although in a manner somewhat distinct from that of crotamine and more similar to  $\text{PLA}_2$  myotoxins. The goal of the current study was to examine the effects of crotamine on a SR preparation containing the  $\text{Ca}^{2+}$  pump and release channel, as well as evaluate the impact of ruthenium red, a calcium release channel blocker, on crotamine action. SR was prepared from guinea pig, human, and horse skeletal muscle. Measurements of calcium uptake into the SR were performed by dual-wavelength spectrophotometry using antipyrilazo III at  $\Delta A$  710-790nm (Toxicon 32 273-278, 1994). Our data suggests that cardiotoxin (10  $\mu\text{M}$ ) either stimulates  $\text{Ca}^{2+}$  uptake or blocks  $\text{Ca}^{2+}$  release in guinea pig SR by 55% ( $p < .005$ ,  $n=3$ ) while crotamine (10  $\mu\text{M}$ ) reduces uptake in human, guinea pig, and horse SR by 71% ( $p < .001$ ,  $n=5$ ). Additionally, it was found that the inhibition of uptake by crotamine could be totally reversed by exposing SR to ruthenium red (3  $\mu\text{M}$ ), a calcium release channel antagonist, prior to myotoxin administration. These results suggest that the predominant site of action of crotamine is at the calcium release channel ( $\text{r}^{\text{r}}\text{r}1$ ) in SR. Specifically, crotamine increases  $\text{Ca}^{2+}$  efflux from  $\text{r}^{\text{r}}\text{r}1$  *in vitro*, with no apparent effects on  $\text{Ca}^{2+}$ -pump activity.

PROPORTIONAL DECREASE OF SARAFOTOXIN SYNTHESIS  
FOLLOWING EMPTYING OF THE VENOM GLAND OF  
*ATRACTASPIS ENGADDENSIS*

A. Bdolah, S. Kinamon and E. Kochva

Department of Zoology, George S. Wise Faculty of Life  
Sciences, Tel Aviv university, Tel Aviv 69978, Israel

The sarafotoxins are a unique group of highly toxic 2500 dalton peptides, that constitute about 30-40% of the proteins of *Atractaspis engaddensis* venom. Most other components are larger proteins with molecular sizes at the range of 30,000 to 120,000 daltons. The venom secreted into the gland lumina during the first three days after emptying of the gland (by multiple strikes) shows a considerably lower proportion of sarafotoxins and a lower toxicity as compared to venom collected after longer intervals. The rosary-type structure of the cDNA, which consists of multiple copies of sarafotoxin and linker peptides, may point to the possibility that large sarafotoxin precursors are first secreted and are then being processed to yield the small, mature sarafotoxins. These precursors have not yet been identified in the venom, however. At longer time intervals after emptying of the gland, the proportion of the sarafotoxins as well as the toxicity of the venom returns to normal. Whether the change in the proportion of sarafotoxins in the venom is due to a specific regulation of their translation, intracellular or extracellular processing, will have to be further investigated.

## BRADYKININ POTENTIATING PEPTIDES FROM SNAKE VENOMS AND THEIR SPATIAL STRUCTURES

Luiza A.F. Ferreira, Depto. de Bioquímica & Biofísica,  
Instituto Butantan, São Paulo, Brasil, and  
Gerhard G. Habermehl, Dept. of Chemistry,  
School of Veterinary Medicine, Hannover, Germany.

Kinins are defined as hypotensive polypeptides which contract extravascular isolated smooth muscle preparations, relax the rat duodenum, decrease the arterial blood pressure of mammals, and cause pain. There is evidence that kinins are important in various physiological, and pathological conditions.

Snake venoms from Viperidae, and Crotalidae, possess low molecular peptides of about ten amino acid residues, potentiating the bradykinin activity, and inhibiting the angiotensin converting enzyme. These peptides play an important role in the clinical manifestation of such bites of snakes whose venom contains them, the more as no antibodies are formed because of their short chain length, and therefore cannot be treated with serum.

This is especially important in the case of bites by snakes who do not possess specifically acting toxins, as e.g. Agkistrodon piscivorus. The symptoms following a bite are a synergistic effect of many enzymes and mediators. In this respect, the bradykinin potentiating peptides are substances essential for the understanding of those envenomations.

Three dimensional structures of such peptides will be shown, which might explain their activities.

# THE EFFECTS OF *BOTHROPS ERYTHROMELAS* VENOM (BEV) ON MEAN ARTERIAL BLOOD PRESSURE (MABP) OF ANESTHETIZED DOGS. INVOLVEMENT OF BRADYKININ.

Zappellini, A., Lélis, A., Prado-Franceschi, J. Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, Campinas, São Paulo, Brazil

*Bothrops erythromelas* is a venomous snake restricted to the northeast of Brazil. The aim of the present work was to investigate the effect of BEV on MABP of anesthetized dogs and to assess the involvement of bradykinin in the hypotensive response to this venom. Mongrel dogs (both sexes,  $8.6 \pm 0.7$  kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Left carotid artery and left femoral vein were cannulated for MABP measurement and drug administration, respectively. Lyophilized BEV (25 µg/kg, 0.7 ml, bolus) was administered to animals which had received saline (0.9%, w/v, n=12, control animals), cellulose sulphate (CS, infusion of two doses of 20 mg/kg, 0.5 ml/min, 8 min, n=7) or captopril (2 mg/kg, 0.5 ml/min, 8 min, n=3). The effects of BEV in control and treated animals were recorded 0, 15, 30, 60 and 120 min after venom administration. BEV administration, led 58.8% of the animals to death, due to respiratory arrest. In the remaining animals, BEV induced a biphasic hypotensive response. Immediately after venom administration, MABP decreased  $51.2 \pm 8.1$  mmHg. After a brief and partial recovery, MABP decreased a further  $76.8 \pm 13.2$  mmHg. CS is a polyanionic agent which depletes high molecular weight kininogen, leading to bradykinin release. The first dose (20 mg/kg) of CS induced a large decrease of MABP ( $-90.0 \pm 15.4$  mmHg). A second equivalent dose induced no further change. The immediate decrease in MABP that follows BEV administration was markedly reduced in CS-treated animals ( $-12.0 \pm 4.1$  mmHg,  $p < 0.01$  when compared to control animals). The second decrease in MABP ( $-57.7 \pm 4.6$  mmHg) did not differ from the corresponding values in control animals. The injection of BEV into captopril-treated animals, led to immediate death. Together, these results indicate that the hypotension induced by BEV is probably due to the release of bradykinin.

Support: FAPESP, FAEP/UNICAMP



STUDY OF INFLAMMATORY EVENTS INDUCED BY  
*Bothrops jararaca* VENOM (BjV) IN THE RAT AIR POUCH.

<sup>1</sup> C.F.P. Teixeira; <sup>1</sup> J.W.M. Costa-Cruz; <sup>2</sup> Y. Cury. <sup>1</sup> Laboratório  
de Farmacologia and <sup>2</sup> Laboratório de Fisiopatologia. Instituto  
Butantan, São Paulo, Brazil.

In the present study the BjV-induced inflammatory reaction in the rat air pouch was characterized in terms of vascular permeability, leukocyte influx and eicosanoid (PGE<sub>2</sub>, LTB<sub>4</sub> and TXB<sub>2</sub>) release. Injection of crude BjV (10ug/rat) into male Wistar rat air pouch induced a significant increase of vascular permeability during the first 30 min. In addition, infiltration of leukocytes, mainly polymorphonuclear cells, into the pouch was observed from 4 to 8 h. This was followed by a decrease to normal values around 24 h. Eicosanoids were detected by ELISA in the pouch washes between 1 and 4 h after BjV injection. The contents of PGE<sub>2</sub> and TXB<sub>2</sub> reached a maximum at 1 h. The levels of LTB<sub>4</sub> were significantly high from 1 to 4 h with a maximum at 4 h. These results indicate that the inflammatory reaction induced by crude BjV in rat air pouch is characterized by an early increase of vascular permeability followed by a marked leukocyte influx. Since eicosanoids are important inflammatory mediators, the release of PGE<sub>2</sub> may be relevant for the venom-induced edema, whereas that of both LTB<sub>4</sub> and TXA<sub>2</sub> may contribute to the observed cell infiltration.

## STUDIES ON ANTICOMPLEMENTARY, PROTEOLYTIC AND HEMOLYTIC ACTIVITY OF *VIPERA AMMODYTES* VENOM

<sup>1</sup>S. A. Stanilova, <sup>2</sup>I. B. Matev- and <sup>1</sup>Z. D. Zhelev

<sup>1</sup>Department of Mol. Biology & Immunology, Medical University, 6000 Stara Zagora, Bulgaria, <sup>2</sup>Laboratory of Herpetology, Higher Institute of Veterinary Medicine & Zootechnics, Stara Zagora 6000, Bulgaria

Snake venoms are important sources of toxins with a wide variety of biological activities. It is well established that *Viperidae* venoms possess indirect hemolytic and proteolytic activities, yet the anticomplementary activity is not studied. Crude venom of *Vipera ammodytes* from Southern Bulgaria was fractionated on QAE Sephadex A-50. Seven well expressed fractions were isolated. Composition of fractions was monitored by electrophoresis on polyacrylamide gels and immunoelectrophoresis. Proteolytic, hemolytic and anticomplementary activities were tested in the various fractions and crude venom. Well expressed anticomplementary activity was observed in fractions V, VI and VII. The complement inactivation was found via both classical and alternative pathways. Fraction VII had a further proteolytic activity, while fractions V and VI possessed phospholipase A2 activity. Normal human sera were incubated with anticomplementary fractions and analysed by immunoelectrophoresis. C3 component of complement and conversion of C3 to the  $\beta 1G$  position were detected employing a specific antibody to C3. Studies were therefore performed on the hemolytic properties of anticomplementary fractions, but lysis was not occurred. These results suggest that the mechanisms of anticomplementary activities of *Vipera* venom are different from *Cobra* venom.

PRODUCTION OF A MONOCLONAL ANTIBODY AGAINST  
CRUDE VENOM FROM THE BROAD-BANDED COPPERHEAD  
(*Agkistrodon contortrix laticinctus*)

Lynda A. Ramos and John C. Perez.  
Biology Department, Texas A&M University-Kingsville, Kingsville,  
TX 78363, USA

*Agkistrodon contortrix laticinctus* (broad-banded copperhead) venom is a hemorrhagic venom which is in the family Crotalinae. Venoms in the Crotalinae family are among the most hemorrhagic of snake venoms. The production of monoclonal antibodies is essential in the understanding of antigenic relatedness of venoms. Monoclonal antibodies were produced against crude venom from *Agkistrodon contortrix laticinctus* (Broad-banded Copperhead). One hundred and twenty three hybridoma cells grew and 51 produced antibodies as measured by the ELISA. Twenty four were frozen and further studied. Isoelectric focusing (IEF) was performed with the crude venom and eleven bands were found. Most of these bands were acidic proteins; however, the western blot revealed that most of the monoclonal antibodies produced against crude venom reacted with basic proteins. Of the twenty four different cell lines blotted, twenty of them showed positive reaction with basic venom proteins and two showed positive reaction with the acidic proteins. The most antigenic proteins in the venom from *Agkistrodon contortrix laticinctus*, which is primarily an acidic venom, were the few basic proteins. Monoclonal antibodies, once cloned, will be useful in screening other venoms, purification of venoms by affinity chromatography, and screening recombinant DNA products.

## IMMUNOLOGICAL RELATEDNESS OF VARIOUS SNAKE VENOMS WITH A MONOCLONAL ANTIBODY

Elda E. Sánchez and John C. Pérez.

Biology Department, Texas A & M University-Kingsville, Kingsville,  
Texas, 78363, USA

Monoclonal antibodies can be used to determine relatedness of venom proteins by ELISA. *Crotalus molossus molossus* venom was fractionated using a Waters Protein Pak 5PW-SP column. Each fraction was tested for hemorrhagic activity, in which fraction one exhibited the most hemorrhagic activity. A monoclonal antibody was produced with fraction one. The antibody was used to screen several venom samples of different snake species. The monoclonal (CMM1) reacted with 15 venom samples from seven different species by ELISA. The ELISA showed strong cross-reactivity with *Crotalus b. basiliscus*, *C. m. nigrescens*, *C. m. molossus* #187, #163, #465, #461, #466, #450, #462, *C. m. molossus* ABC Pooled, *C. viridis cerberus* #172, two Elapidae species *Ophiophagus hanna* and *Oxyuranus scutellatus*, and one Viperid species *Psuedocerastis fieldi*. Activity between the monoclonal antibody and *C. m. molossus* #487, #60, #476, #464, #460 were extremely weak, displaying ELISA readings between 0-0.019. A more interesting observation was the antibody activity with one individual species of *C. viridis cerberus* #172 and the lack of activity of another snake of same species (#171) from the same location. Understanding the relatedness of different snake species will allow for efficient production of neutralizing antibodies useful for medical treatment of snakebites and other biomedical applications.

**PARTIAL CHARACTERIZATION AND THE ROLE OF  
GLYCOSYL MOIETIES OF THE HEMORRHAGIC TOXIN,  
PROTEINASE H, FROM *CROTALUS ADAMANTEUS* VENOM**

Steffan G. Anderson and Charlotte L. Ownby

Department of Physiological Sciences, Oklahoma State University, Stillwater,  
OK 74078, U. S. A.

Proteinase H was purified using an open column and HPLC liquid chromatographic procedure modified from the method first reported by Kurecki and Kress in 1985. The protein gave a positive reaction for carbohydrate when stained with periodic acid-Schiff. The 86,000 Dalton hemorrhagic metalloproteinase has a carbohydrate mass content of  $41.3 \pm 2.4$  % *N*-linked carbohydrate as determined by removal of sugar moieties with the glycosidase PNGaseF followed by SDS-PAGE. The nature of the glycosylation was determined by lectin affinity blotting against the toxin and *Datura stramonium* agglutinin (DSA) which is specific for glycosyl residues containing the disaccharide galactose  $\beta(1-4)$  *N*-acetylglucosamine in complex and hybrid *N*-glycans (Crowley, *et al.* 1984). The role of sugar moieties in the proteolytic and hemorrhagic activities of proteinase H were studied. Removal of sugar moieties by PNGaseF or their oxidation by 0.5 M sodium periodate totally abolished the hemorrhagic and proteolytic activity of the toxin. Removal of sialic acid residues by *Vibrio cholerae* neuraminidase had no effect on hemorrhagic activity but resulted in a decrease in proteolytic activity of  $86.83 \pm 8.9$ %. These data indicate that the glycosyl moieties of proteinase H, in their intact ring formations, are essential to the enzymatic and hemorrhagic activity of the toxin, probably functioning in stabilization of the tertiary structure of the protein and possibly in ligand/target site binding by serving as recognition sites or ligands for the target site of the toxin.

Crowley, J. F., Goldstein, I. J., Arnarp, J. and Lonngren, J. (1984) *Arch. Biochem. Biophys.* 231, 524.

Kurecki, T and Kress, L. F. (1985) *Toxicon* 23(4), 657.

# SCREENING A cDNA LIBRARY FROM *Crotalus atrox* WITH MONOCLONAL ANTIBODIES SPECIFIC FOR HEMORRHAGIC TOXINS IN VENOM

Sonia K. Lerma, Albar Chavana, and James R. Pierce. Texas A&M University-Kingsville, Kingsville, TX 78363

The venom of the western diamondback rattlesnake (*Crotalus atrox*) is a complex mixture of toxins. Hemorrhagic toxins, the predominant toxins, are zinc metalloproteinases which cause extensive tissue damage in animals and humans. A cDNA library has been constructed from *C. atrox* venom glands and screened with monoclonal antibodies specific to hemorrhagic toxins from *C. atrox*. The screening technique identifies clones expressing hemorrhagic genes and is therefore a functional assay. Because of the potential lethality to the host of the cloned zinc metalloproteinases, the cDNAs were transformed into *E. coli* Top 10 F', an expression vector which has an up-promoter mutation in the *lacI* gene. The resulting overproduction of repressor together with the induction of catabolite repression by the addition of glucose effectively inhibits transcription of the cDNA. Restriction digests indicate that the size of the inserts range from 1 kb to 2.2 kb. Nitrocellulose colony lifts from the library were overlaid on Luria-Bertani plates with ampicillin and isopropyl- $\beta$ -D-thiogalactoside but without glucose. The colonies were allowed to grow for three hours in order to express the cloned genes. Approximately five thousand recombinants were screened with a mixture of three monoclonal antibodies specific to *C. atrox* hemorrhagins. Nine colonies that reacted positively have been characterized and the cloned DNA will be sequenced and compared to published sequences. The library is being reprobbed with oligonucleotides homologous with published sequences to identify nonexpressing clones.

# THE STRUCTURE AND THE FUNCTION OF MONGOOSE ANTIHEMORRHAGIC FACTOR

<sup>1</sup>H. Yanai and <sup>2</sup>T. Tomihara

<sup>1</sup>Department of Bioscience and Biotechnology,  
University of the Ryukyus, Nishihara, Okinawa 903-01  
Japan

<sup>2</sup>Department of Habu, Okinawa Prefectural Institute of  
Public Health, Ozato, Okinawa 901-22, Japan

There are three antihemorrhagic factors (AHF1, AHF2 and AHF3) in the serum of mongoose (Herpestes edwardsii). The structure of one of them, AHF1, has been examined protein-chemically, and subjected to a homology search with those of other proteins. The amino acid sequences of thirteen peptides isolated from AHF1 were found to be homologous to one of the five domains of human  $\alpha$ 1B-glycoprotein ( $\alpha$ 1B3), including cysteine residues forming disulfide linkages and one of the sugar binding sites of human  $\alpha$ 1B3. Thus, the mongoose antihemorrhagic factor may be a supergene family of immunoglobulins.

AHF1 forms a weak complex with HR2b, a hemorrhagic factor of Habu snake (Trimasurus flavoviridis). There is no alteration in the primary or the secondary structure of both factors before and after complex formation. In addition the activities of both factors were fully recovered by dissociation of the complex.

These results suggest that the molecular mechanism of the inhibition of the hemorrhagic activity of HR2b by AHF1 in vivo is different from that of a conventional protease inhibitor, but similar to that of antigen-antibody interaction in immune system.

## A SNAKE VENOM DISINTEGRIN PREVENTS REOCCLUSION FOLLOWING THROMBOLYSIS IN A CANINE CAROTID ARTERY THROMBOSIS MODEL AND BLOCKS MELANOMA METASTASIS

F.S. Markland, Dept. of Biochemistry & Molecular Biology, University of Southern California, School of Medicine, Los Angeles, CA 90033 U.S.A.

Contortrostatin, a disintegrin purified from southern copperhead snake venom, is a disulfide linked homodimer with a molecular mass of 13,510 daltons. Contortrostatin blocks integrins of the  $\beta_1$  and  $\beta_3$  subclasses and inhibits platelet aggregation by binding to the fibrinogen receptor (integrin GPIIb/IIIa). We examined the effect of contortrostatin on reocclusion after thrombolysis in a canine carotid artery thrombosis model. The right carotid artery was instrumented with a flow probe, a mechanical constrictor, and an electrode which was used to produce an occlusive thrombus. Following 30 minutes of continuous occlusion of the carotid artery, APSAC (acylated plasminogen-streptokinase activator complex) was administered locally to induce thrombolysis. Immediately after APSAC, saline ( $n=6$ ) or contortrostatin (0.15 mg/kg,  $n=7$ ) was administered intravenously. Saline-treated animals reoccluded within  $41 \pm 8$  minutes. None of the contortrostatin-treated animals reoccluded. Heart rate and blood coagulation parameters were not changed in the contortrostatin-treated animals. A transient decrease in platelet count was observed. *Ex vivo* platelet aggregation in response to ADP or arachidonic acid was inhibited by contortrostatin and bleeding time was increased. However, the bleeding time returned almost to the baseline value upon completion of the experiment and the platelet count returned to normal. Thrombus weight was significantly reduced in the contortrostatin group ( $20.4 \pm 4$  mg) versus saline-treated controls ( $48 \pm 8$  mg). In conclusion, contortrostatin effectively prevents reocclusion in the canine arterial thrombosis model. Separately, we observed that contortrostatin inhibited binding of metastatic human melanoma (M24met) cells to immobilized collagen type I, vitronectin, and fibronectin ( $IC_{50}=20, 75$ , and  $220$  nM, respectively). We also found that contortrostatin inhibits lung colonization of intravenously introduced M24met cells in SCID mice. Pretreatment of the tumor cells with contortrostatin ( $100 \mu\text{g}/5 \times 10^5$  cells) inhibited by  $\sim 75\%$  the number of melanoma cells forming colonies in the lungs. Supported in part by AHA-GLAA Grant # 938 GI-1 and by NIH Grant # CA54861.



# PURIFICATION AND CHARACTERIZATION OF ANTICOAGULANTS FROM *AUSTRELAPS SUPERBUS* (COPPERHEAD) SNAKE VENOM

R. Manjunatha Kini  
Bioscience Centre, Faculty of Science  
National University of Singapore, Lower Kent Ridge  
Road, Singapore 0511

Snake venoms are complex mixtures of pharmacologically active proteins and polypeptides. Several snake venoms and purified proteins from these venoms inhibit blood coagulation and platelet aggregation. Procoagulant activities of Australian snake venoms are well studied, but the anticoagulant proteins from these venoms have not yet been characterized. I have purified two new anticoagulant proteins, superbin I and superbin II, from *Austrelaps superbis* venom by gel filtration and ion-exchange chromatographic methods. These anticoagulants inhibit both blood coagulation and platelet aggregation. They also exhibit phospholipase A<sub>2</sub> activity. Preliminary studies indicate that both praelongins inhibit the activities of extrinsic tenase and/or prothrombinase complexes. Recently, similar anticoagulant proteins have been isolated and characterized from *Pseudechis australis* (king brown snake) (P. Mirtschin and P. Massey, personal communications and unpublished observations) and *Acanthophis praelongus* (northern death adder) (unpublished observations) venoms.

I thank Mr. Sivan Subburaju for his excellent technical assistance. This work is supported by a financial grant from the Economic Development Board of Singapore and an academic research grant (RP 940325) from the NUS.

PURIFICATION AND MOLECULAR CLONING OF  
THROMBIN-LIKE ENZYME FROM THE VENOM OF  
*AGKISTRODON CALIGINOSUS* (Korean Buldogsa)

<sup>1</sup>Yeong-Shik Kim, <sup>1,2</sup>Kyoung-Youl Yang, <sup>2</sup>Eun-Mi Choi,  
<sup>1</sup>Bum-Soo Hahn and <sup>1</sup>Il-Moo Chang,

<sup>1</sup>Natural Products Research Institute, Seoul National University,  
Seoul, 110-460, Korea

<sup>2</sup>Dept of Chemistry, University of Incheon, Incheon, 402-749, Korea

The thrombin-like enzyme has been purified from the venom of *Agkistrodon caliginosus* using conventional chromatography including Bio-Gel P-100, Mono S HR 5/5 and Pro-RPC columns. The enzyme was a monomer with a molecular weight of 34,000 by SDS-PAGE and its isoelectric point was 5.8. The enzyme acted on fibrinogen to form fibrin and specific to chromogenic substrate, T1637. This enzyme contained abundant asparagine or aspartic acid, and there was little tyrosine or methionine. The protease showed higher activity toward TAME than thrombin but had no caseinolytic activity. Enzyme activity was strongly inhibited by PMSF, moderately by benzamidine and soybean trypsin inhibitor, indicating it is a serine protease inhibitor. The 1.5 kb cDNA for the enzyme was cloned from a  $\lambda$ ZAP cDNA library derived from the venom glands. The amino acid sequence of the enzyme exhibited significant homology to those of mammalian serine proteases (trypsin, pancreatic kallikrein and thrombin) and other thrombin-like enzymes such as ancrod, batroxobin and flavoxobin. (Supported by Genetic Engineering Grant from the Ministry of Education)

## **FIBROLASE, AN EFFECTIVE THROMBOLYTIC AGENT IN ARTERIAL AND VENOUS THROMBOSIS ANIMAL MODELS**

F.S. Markland, Dept. of Biochemistry & Molecular Biology, University of Southern California, School of Medicine, Los Angeles, CA 90033 U.S.A.

Fibrolase is a fibrinolytic metalloproteinase of 23 kDa isolated from southern copperhead snake venom. The enzyme acts directly on fibrin clots and does not require plasminogen or any other blood-borne intermediates for activity. The enzyme is not inhibited by blood serine proteinase inhibitors. In collaboration with J.J. Bookstein, University of California, San Deigo, we showed that the enzyme lyses acute clots in the renal arteries of rabbits and subacute clots in rabbit iliac veins. There was no histological or gross evidence of hemorrhaging and no side effects were observed. Recently, in collaboration with B.R. Lucchesi, University of Michigan, we investigated the efficacy of recombinant fibrolase to lyse a thrombus formed in the carotid artery of the dog. Five anesthetized dogs were instrumented for the measurement of systemic pressure and right/left carotid artery blood flow velocity. Electrolytic injury was initiated in both the right and left carotid artery. Thirty minutes after both arteries were occluded, fibrolase (4 mg/kg in 3 ml) was infused proximal to the thrombus in the left carotid artery only. Physiological saline (identical rate as fibrolase infusion) was infused proximal to the thrombus in the right carotid artery, simultaneously. In the artery infused with fibrolase 5 out of 5 dogs exhibited reflow within  $6 \pm 1$  minutes of infusion ( $p < 0.05$ ) vs vehicle treated artery (Fisher's Exact test). In the corresponding arteries that received vehicle, the occlusion was maintained in each dog throughout the experimental protocol. Five minutes after the end of fibrolase infusion, a GPIIb/IIIa antibody, 7E3 (0.8 mg/kg i.v.), was administered to prevent reocclusion of the patent artery. In 4 out of 5 dogs the vessel treated with fibrolase followed by 7E3 administration remained patent for the entire 2 hour observation period. Our findings indicate that the administration of fibrolase at the site of an occlusive arterial or venous thrombus can achieve rapid thrombolysis and restoration of blood flow. In the carotid arterial model, rethrombosis occurs in the absence of adjunctive therapy and can be prevented by the administration of a platelet glycoprotein IIb/IIIa receptor antibody, 7E3. Supported in part by NIH Grant # HL31389. Recombinant fibrolase was provided by Chiron Corp., Emeryville, CA.

**FIBRINOGEN-CLOTTING ACTIVITY OF PROTEINASE H, THE  
HEMORRHAGIC ZINC-METALLOPROTEINASE FROM  
*CROTALUS ADAMANTEUS* (EASTERN DIAMONDBACK  
RATTLESNAKE) VENOM**

Steffan G. Anderson and Charlotte L. Ownby

Department of Physiological Sciences, Oklahoma State University, Stillwater,  
OK 74078, U. S. A.

Proteinase H, the sole hemorrhagic factor in the venom of the Eastern diamondback rattlesnake, was first isolated from crude venom by Kurecki and Kress in 1985. In the current study, proteinase H was isolated in a simplification of the method published by Kurecki and Kress and was found to be a fibrinogen-clotting enzyme. To our knowledge this is the first time such activity has been reported for a snake venom hemorrhagin. The fibrinogen-clotting activity of proteinase H was quantified by monitoring the increase in turbidity of bovine fibrinogen in aqueous solution at 445 nm. The effects of EDTA, PMSF and deglycosylation with PNGaseF on the fibrinogen-clotting activity of proteinase H were investigated. Fibrinopeptide release was determined by RP-HPLC and the structure of fibrin clots formed by proteinase H treatment investigated by transmission electron microscopy. The results of this study indicate that proteinase H is a fibrinogen-clotting enzyme which causes the polymerization of bovine fibrinogen into fibrin strands that are similar in appearance to those formed by treatment with thrombin.

Kurecki, T. and Kress, L. F. (1985) *Toxicon* 23(4), 657.

## FIBRIN MONOMER GENERATION AND THEIR POLYMERIZATION BY *VIPERA RUSSELLI* VENOM.

ABID AZHAR, FATIMA SUGHRA AUSAT, AND FIZZA AHMAD  
Coagulation and Haemostasis Research Unit, Department of Biochemistry,  
University of Karachi, Karachi-75270, Pakistan

Snake venoms have long been known to possess proteins that can affect blood coagulation. Some of these proteins are directly involved in fibrinogen-fibrin conversion. Moreover, due to their significantly narrow specificity, they can exclusively cleave either fibrinopeptide A or fibrinopeptide B. This allows their use as chemical probes to study the kinetics of formation and characteristics of fibrin networks.

In the current investigation, human plasma fibrin networks were developed using crude venom from *Vipera russelli* and the kinetics of development of the networks was followed. The network characteristics were compared with those of the bovine thrombin induced networks. Turbidimetric studies showed that the turbidity of the networks developed from venom was significantly lower than that of networks developed using bovine thrombin. The networks developed in the presence of  $\text{CaCl}_2$  were made up of thicker fibres than those developed with the venom alone. Repolymerization studies with venom generated monomers also produced thinner fibres than those of thrombin generated monomers. This difference between the networks developed with venom and those developed with bovine thrombin is enhanced upon increase in the protein concentration. SDS-PAGE electrophoresis revealed characteristic bands of  $\alpha$ ,  $\beta$ , and  $\gamma$  in both venom and thrombin induced networks.

EFFECT OF *BOTHRUPS JARARACA* (Bj) AND *CROTALUS DURISSUS TERRIFICUS* (Cdt) VENOM ON NEUTROPHIL MIGRATION. *IN VITRO* CHEMOTAXIS STUDIES.

S.H.P.Farsky, L.R.C.Gonçalves, M.C.C.Sousa e Silva and Y.Cury.  
Laboratório de Fisiopatologia, Instituto Butantan, São Paulo, Brasil.

While the accident evoked by Bj venom is characterized by inflammatory response with purulent exudate, this is not observed in Cdt envenomation. Since the mechanisms involved in the cellular aspects of these phenomena are unknown, the study of the effects of Bj and Cdt venoms on the mechanisms of oriented neutrophil migration (chemotaxis) was a first approach to investigate this subject. Experiments were performed using a multiwell chamber (modified Boyden's chamber). Serum and neutrophils were obtained from male Wistar rats. Bj and Cdt venoms doses ranged between 10 to 200 µg/ml and 1 to 10 µg/ml, respectively. Results showed that: 1) preincubation of neutrophils with the venoms did not alter the ability of the cells to respond to lipopolysaccharide from *Escherichia coli* (LPS) activated serum; 2) the venoms did not induce neutrophil oriented locomotion, 3) Cdt venom did not generate chemotactic factor derived from serum as opposed to the positive effect of Bj venom. The migration evoked by 100 and 200 µg/ml of Bj venom was equivalent to that obtained with LPS-activated serum. Complement-depleted serum (pre-heated serum, 56°C, 1 hour) did not evoke neutrophil migration. The *in vitro* results obtained with Cdt venom corroborate the effects observed in the South American rattlesnake-induced envenomation. On the other hand, the neutrophil migration induced by the interaction of only high doses of Bj venom and serum seems to be mediated by complement system.

PARTICIPATION OF ENDOGENOUS CORTICOSTEROIDS IN  
ACUTE INFLAMMATION (EDEMA) INDUCED BY *BOTHROPS*  
*JARARACA* VENOM (BjV).

<sup>1</sup>Y.Cury, <sup>1</sup>S.H.P.Farsky and <sup>2</sup>C.F.P.Teixeira. <sup>1</sup>Laboratório de  
Fisiopatologia and <sup>2</sup>Laboratório de Farmacologia, Instituto Butantan,  
São Paulo, Brazil.

It is well known that endogenous corticosteroids (EC) are important modulators of inflammatory responses. The present study was undertaken to investigate the potential EC regulation of BjV-induced edematogenic response. Male Wistar rats were tested for their capacity to respond to the venom injected into the subplantar area of hindpaws (5 µg/paw). These animals were divided into 4 groups: 1) venom injected into one paw and saline - into the other of either adrenalectomized, sham-operated or normal rats; 2) normal rats injected simultaneously with the venom into both paws; 3) one paw of normal rats injected with BjV and the other one injected 45 min or 2.5 hr later; 4) normal rats injected with the venom only into one paw. At different intervals after BjV injection, the volume of the paws was estimated by plethysmography. The results showed that the edematous response induced by BjV is similar in the adrenalectomized animals as compared to controls. Furthermore, simultaneous or separated injections of the venom into the hindpaws of rats resulted in edematous reactions of comparable time-course development and analogous intensity. These data suggest that endogenous corticosteroids are not relevant factors in modulating BjV-induced inflammation.

ENVENOMATION BY SNAKES OF THE GENUS *Bothrops*  
IN RECIFE - BRAZIL

<sup>1</sup>M.C. Guarnieri, <sup>1</sup>L.R. Andrade, <sup>1</sup>P.K. Lima, <sup>1</sup>D.C.C. Pimentel, <sup>1</sup>K.C. Maia,  
<sup>1</sup>C.M.L., Vasconcelos, <sup>1</sup>R.C. Valença, <sup>2</sup>M.L. Porto and <sup>2</sup>A.E. Oliveira Jr.  
<sup>1</sup>Depto. de Biofísica e Radiobiologia - Universidade Federal de Pernambuco -  
Recife - Brasil, <sup>2</sup>CEATOX - Hospital da Restauração - Recife - Brasil.

Bothropic accidents are responsible by 80% of snake envenomation in Brazil but in Recife they represent only 47% of poisonous snake bite. We report here a retrospective study these 55 cases that were treated at Restauração Hospital - Toxicological Accompany Center during the two and half years period. The most frequent clinical manifestation were: local pain (89.1%), local edema (85.4%), hemorrhage disturbs (47.3%), hyperemia (31%) and cutaneous lesion (25.4%). Among the 42 patients in which blood clotting time was measured, 20% had prolonged coagulation time and 34.5% had blood incoagulability. The alteration of urine color was detect only in 16.4% of the cases. The distribution according to the gravity of the accidents was mild (38.2%), moderate (40%) and serious (21.8%). The bothropic envenomation was responsible by all deaths (3 cases) occurred by poisonous animals. Only 5.5% of the patients were attended within the first hour, 18.2% up to six hours and 69% within six to forty eight hours. The majority of the victims received serotherapy: 1-10 ampoules of antivenotrophic or polyvalent serum (47.3%), 11-20 ampoules (27.3%) and more than 20 ampoules (5.4%). Although the relative number of accidents by genus *Bothrops* is lower than observed in Brazil, the gravity these cases and the importance of treatment at an early stage of envenomation are emphasized.

Supported by CNPq-PROPESQ, FACEPE



POISONOUS ANIMALS ACCIDENTS IN PERNAMBUCO, BRAZIL:  
A EPIDEMIOLOGICAL RETROSPECTIVE STUDY FROM 1992 TO 1994

<sup>1</sup>M.C. Guarnieri, <sup>1</sup>D.C.C. Pimentel, <sup>1</sup>K.C. Maia, <sup>1</sup>L.R. Andrade, <sup>1</sup>P.K. Lima,  
<sup>1</sup>C.M.L. Vasconcelos, <sup>1</sup>R.C. Valeça, <sup>2</sup>M.L. Porto and <sup>2</sup>A.E. Oliveira Jr.

<sup>1</sup>Depto. de Biofísica e Radiobiologia - Universidade Federal de Pernambuco -  
Recife - Brasil, <sup>2</sup>CEATOX - Hospital da Restauração - Recife - Brasil.

The Federal University of Pernambuco together with the Toxicological Accompany Center (CEATOX), initiated in 1992 regional studies of poisonous animals in the Northeast of Brazil. Based on the CEATOX records, a total of 754 cases classified according to the clinical aspects were analyzed. Among them, were registered 48.7% of the accidents by arachnids, 40.3% by snakes, 4.1% by insects, 2.6% by miriapodes and 1.4% by aquatic animals. All accidents involving poisonous animals occur uniformly throughout the year, without seasonal variation as observed in Southeast of the country being the feet and hand the most attacked anatomical area. The poisoning snake accidents were caused predominantly by the genus *Crotalus* (52.1%) and *Bothrops* (47.0%). These snakebites occurred primarily during the day (64%), in rural areas (76.1%), involved mainly males (82.9%) with no apparent predominance of a special age group. Almost the totality of arachnid accidents was by scorpions (98.4%) being the accidents preferentially occurred during the night (36.2%), came from urban areas (89.8%), in children (27.4%) with no predominance of the victim sex. The authors detach the high number of crotalic and scorpionic accidents in relation to the rest of Brazil.

Supported by: CNPq-PROPESQ, FACEPE

**NEUTRALIZING CAPACITY OF SPECIFIC AND COMMERCIAL ANTISERA AGAINST *Crotalus durissus terrificus* AND *Bothrops jararacussu* VENOMS AND THEIR MAJOR TOXINS (CROTOXIN AND BOTHROSPROTOXIN)**

Franco, Y.O., Prado-Franceschi, J. and Rodrigues-Simioni, L.

Department of Pharmacology, Faculty of Medical Sciences, PO Box 6111, UNICAMP, 13084-100, Campinas, SP, Brazil.

The ability of specific and commercial antisera to neutralize the neuromuscular blocking activity of *C. durissus terrificus* and *B. jararacussu* venoms and of their major toxins (crotoxin and bothroprototoxin, respectively) was studied *in vitro* using the mouse phrenic nerve-diaphragm preparation. Specific antisera were prepared by successively inoculating rabbits with the desired venoms over a period of two months (Ownby *et al.*, *Toxicon*, **28**:189, 1990) 1979). The antibody titer was determined by Ouchterlony immunodiffusion throughout the immunization process. The protective effect of the antisera was assessed by pre-incubating them for 30 min with their corresponding venom and toxin and then determining the 50% paralysis time corresponding to an equivalent reduction in the twitch-tension. The degree of protection against the initial facilitation (pre-synaptic effect) induced by *C. d. terrificus* venom and crotoxin was also determined. The control 50% paralysis times were  $88 \pm 2$  min for *C. d. terrificus* venom (20  $\mu\text{g/ml}$ ,  $n=6$ ),  $70 \pm 0.4$  min for crotoxin (10  $\mu\text{g/ml}$ ,  $n=3$ ),  $100 \pm 0.6$  min for *B. jararacussu* (50  $\mu\text{g/ml}$ ,  $n=3$ ) and  $37 \pm 0.7$  min for bothroprototoxin (20  $\mu\text{g/ml}$ ,  $n=3$ ). In all cases, both the specific and commercial antisera preserved the normal responses of the preparations for up to 120 min. The antisera also abolished the pre-synaptic effects of *C. d. terrificus* venom and crotoxin.

Financial support: CAPES and FAEP/Unicamp

CLINICAL ASPECTS AND TREATMENT OF ACCIDENTS  
BY GENUS *Crotalus*

<sup>1</sup>M.C. Guarnieri, <sup>1</sup>K.C. Maia, <sup>1</sup>L.R. Andrade, <sup>1</sup>P.K. Lima, <sup>1</sup>D.C.C. Pimentel,  
<sup>1</sup>R.C. Valença, <sup>1</sup>C.M.L. Vasconcelos, <sup>2</sup>M.L. Porto and <sup>2</sup>A.E. Oliveira Jr.  
<sup>1</sup>Depto. de Biofísica e Radiobiologia - Universidade Federal de Pernambuco -  
Recife - Brasil, <sup>2</sup>CEATOX - Hospital da Restauração - Recife - Brasil.

The present work details a retrospective study of 61 crotalic accidents attended at the Restauração Hospital and Toxicological Accompany Center (CEATOX) in Recife from April 1992 to December 1994. The diagnosis was established by a symptomatologic clinical criterion. The distribution according to the time between the accident and the health care receive by the patients was only 3.2% within the first two hours and 21.3% within the first six hours. The majority of the victims (42.6%) was attended within the six to twenty four hours. The most frequent signs and symptoms were palpebral ptosis (64%) followed by troubled vision (59%). In 47.5% of the cases was observed myalgia, mydriases, alteration of urine color and edema. Among the renal disturbs were detected oliguria (16.4%), anuria (8.2%), increase of the creatinine (9.8%) and urea levels (13.1%) resulting in acute renal failure. The blood clotting time was altered in 8.2% of the victims. Among the cases 59% were considered moderate and 41% severe. The serotherapy treatment was given in almost the totality of the patients and two of them needed hemodialysis. Approximately 50% of the victims received 10 to 19 ampoules of anticrotalic or polyvalent serum, 34.4% received 20 to 29 ampoules and 12.6% more than 30 ampoules. Despite the considerable delay before victims reach hospital and the number of serious accidents, no death were registered and in all cases there was complete recovery from the above symptoms.

Supported by: CNPq / PROPESQ, FACEPE

## NEW APPROACHES IN ANTIVENOM THERAPY

F. Audebert<sup>(1)</sup>, C. Bon<sup>(1)</sup>, V. Choumet<sup>(1)</sup>, G. Rivière<sup>(1)</sup>,  
A. Robbe-Vincent<sup>(1)</sup>, M. Sorkine<sup>(1)</sup>, A. Sabouraud<sup>(2)</sup>,  
J.-M. Scherrmann<sup>(2)</sup> & M. Urtizberea<sup>(2)</sup>

(1) Unité des Venins, Institut Pasteur, Paris, France &

(2) Unité INSERM U26, Hôpital Fernand Widal, Paris, France.

In France, 1000 to 2000 viper bites are recorded each year with a mortality of 0.5%. In order to be effective, the treatment has to be undertaken precociously, but for clinicians, the prediction of the course of envenoming is difficult. A fast ELISA able to detect the venom in blood and urine in less than 40 min was set up and used to quantify venom in blood and in urine samples from patients bitten by vipers. A good correlation was established between the severity of the clinical symptoms and the level of the venom in patient blood.

Toxicokinetics study of *Vipera aspis* venom were performed in rabbits after experimental envenomations. Parameters obtained after intravenous injection of the venom showed that: i) most of the injected dose is eliminated three days after the administration (half-life of elimination: 12 hours); ii) the venom presents a large volume of distribution; iii) the total clearance of the venom is low. The toxicokinetic of the venom administered intramuscularly revealed a precocious resorption of the venom from the site of the injection but the resorption process persisted up to 72 hours after the injection, maintaining a high concentration of venom antigens in the plasma. A quantitative analysis indicated that 65% of the dose injected intramuscularly is able to reach the blood circulation. Similar investigations performed after administration of antivenom allowed to analyse *in vivo* the efficacy of serotherapy. These studies indicated that the intravenous administration of specific anti-venom antibodies (Fab'2) causes the redistribution of the venom antigens from tissues to vascular compartment, where they are efficiently neutralized and eliminated.

These observations proved to be very useful for a better rationalization of the immunological treatment of envenomations.

## MTx1 AND 2 FROM GREEN MAMBA VENOM ALLOSTERICALLY ACTIVATE M1 AND BLOCK M3 MUSCARINIC RECEPTORS.

<sup>1</sup>D. Jerusalinsky, <sup>1</sup>E. Kornisiuk, <sup>2</sup>S. Habtemariam and <sup>2</sup>A. Harvey.

<sup>1</sup>IBCN, Fac. Med., Univ. Buenos Aires, 1121 Bs. As., Argentina. <sup>2</sup>Dept. Physiol. and Pharmacol., SIDR, Univ. Strathclyde, Glasgow G1 1XW, UK

MTx1 and 2, 7500 Da proteins from Eastern green mamba snake (Dendroaspis angusticeps) venom, specifically bind to m<sub>1</sub> and m<sub>3</sub> cloned human muscarinic receptors (MACHR)<sup>a</sup>. On rabbit vas deferens, MTx1 reduced the twitch response to neural stimulation, as the m<sub>1</sub> agonist McN-A-343 does. Pirenzepine or atropine did not prevent the effect of the toxins, but they block the effect of McN-A-343<sup>b</sup>. Similar results have been obtained with MTx2. This work was carried out to further characterize the binding properties and functional effects of MTx1 and 2, using isolated muscle preparations that contain M1, M2 and M3 pharmacological subtypes of MACHR (rabbit vas deferens, guinea pig atria and ileum, respectively). Neither MTx1 nor MTx2 had any direct action on guinea pig atria or ileum. PBCM, an irreversible blocker of MACHR, did not prevent the action of MTx on vas deferens. Preincubation with MTx of m<sub>1</sub> cloned receptors prevented the binding of <sup>3</sup>H-NMS, suggesting that they were irreversibly bound. But <sup>3</sup>H-PBCM still bound to membranes preincubated with MTx. The allosteric modulators of MACHR, gallamine and tacrine, modified the effects of MTx1 in rabbit vas deferens. Muscarinic agonists activate PI metabolism on SK-N-SH neuroblastoma cells, which express m<sub>3</sub> receptors. Neither MTx1 nor MTx2 had any direct effect, but they reduced the methacholine-induced increase in PI turnover. In conclusion, MTx1 and 2 activate M1 receptors at a site different from that of the agonists, which is blocked by PBCM, while MTx1 and 2 behave as antagonists at M3 receptors.

a. Kornisiuk et al., Toxicon 1995 (in press).

b. Jerusalinsky et al., Life Sci 1995 (in press).

A SENSITIVE AND SPECIFIC IN-SITU  
MODEL TO STUDY THE PHARMACOLOGICAL  
MECHANISMS OF AGENTS/ TOXINS

J. von Bredow, J. Vick, H. Lowensohn, and N. Adams  
Walter Reed Army Institute of Research, Wash. D.C.

Many biological warfare agents intoxicate both vital central and peripheral organ systems causing death from multiple unexplainable causes. Biological toxins often cause death from respiratory cessation. Previous studies have shown that many venoms stop impulse transmission at the neuro-muscular junction even as the peripheral nerves continue to fire. Systemic toxins often involve other organ systems (CNS, cardiovascular, renal) which leads to confusion in assessing the most appropriate treatment regimen or antidote. In an attempt to correct for these confusing results an in-situ model has been developed to accurately characterize the effects of toxins in a physiologically functioning gastrocnemius muscle preparation. The sciatic nerves of anesthetized rabbits are isolated and stimulated to produce contractions of the gastrocnemius muscles. Toxic solutions are administered directly to the muscle mass by direct injection into a cannulated branch of the femoral artery. This allows for the administration of minute quantities of test substances into the leg without exposing the whole animal. This also allows other vital organ systems to maintain normal physiological functions even as the effect of the toxin is being observed in the hindlimb. It is felt that this preparation provides a specific and sensitive model for studying the direct effects of neurotoxins on the neuromuscular junction, without systemic involvement.

## KINETIC ISOTOPE EFFECT STUDIES ON THE TRANSITION STATE OF PERTUSSIS TOXIN

J. Scheuring and V.L. Schramm

Department of Biochemistry, Albert Einstein College of Medicine  
Bronx, NY 10461, U.S.A.

Pertussis toxin catalyzes the transfer of the ADP ribosyl moiety from  $\text{NAD}^+$  to a specific cysteine residue of several G-proteins, four amino acids from the C-terminus. In absence of an acceptor protein, pertussis toxin acts as  $\text{NAD}^+$  glycohydrolase. Primary and secondary kinetic isotope effects were determined for the hydrolysis reaction, using  $^3\text{H}$ ,  $^{14}\text{C}$  and  $^{15}\text{N}$  as atomic labels. Primary kinetic isotope effects were  $1.026 \pm 0.005$  for  $[\text{C}_\text{N}1'\text{-}^{14}\text{C}]$  and  $1.031 \pm 0.005$  for  $\text{N}_\text{N}\text{-}^{15}\text{N}[\text{NAD}^+]$ . A primary double kinetic isotope effect was determined as  $1.057 \pm 0.002$  for  $[\text{C}_\text{N}1'\text{-}^{14}\text{C}, \text{N}_\text{N}\text{-}^{15}\text{N}]\text{NAD}^+$ . Secondary kinetic isotope effects were  $1.182 \pm 0.014$  for  $[\text{C}_\text{N}1'\text{-}^3\text{H}]\text{NAD}^+$ ,  $1.144 \pm 0.006$  for  $[\text{C}_\text{N}2'\text{-}^3\text{H}]\text{NAD}^+$ ,  $0.994 \pm 0.004$  for  $[\text{C}_\text{N}4'\text{-}^3\text{H}]\text{NAD}^+$  and  $1.019 \pm 0.004$  for  $[\text{C}_\text{N}5'\text{-}^3\text{H}]\text{NAD}$ . These data indicate a strong oxocarbenium transition state for the toxin. Kinetic isotope effects for the pertussis toxin transfer reaction were performed using a synthetic peptide as a model acceptor. The peptide corresponds to the last 20 amino acids of the  $\text{G}_{13}$  protein. The experiments showed similar isotope effects for most of the atomic labels relative to the hydrolysis reaction. A significant difference was found for the  $[\text{C}_\text{N}2'\text{-}^3\text{H}]\text{NAD}^+$ , which was  $1.080 \pm 0.007$  and a slightly increased value in the case of the  $[\text{C}_\text{N}1'\text{-}^{14}\text{C}]$ ,  $\text{NAD}^+$  ( $1.034 \pm 0.004$ ). Participation of the cysteine nucleophile may have an effect on the nature of the transition state of the transfer reaction. The product of the transfer reaction shows  $\alpha$ -configuration for the  $\text{C}_\text{N}1'$  carbon by NMR spectroscopy. Thus, the reaction occurs by inversion of configuration.

Supported by NIH grant AI34342-03.

**Determination of the Minimum Cytotoxic *Clostridium perfringens* Enterotoxin Fragment through Deletion Analysis.**

JOHN F. KOKAI-KUN\* and BRUCE A McCLANE, University of Pittsburgh School of Medicine, Pittsburgh PA.

To study the structure/function relationship of *Clostridium perfringens* enterotoxin (CPE), full-length CPE (CPE<sub>1-319</sub>), six N-terminal CPE truncation fragments (CPE<sub>37-319</sub>, CPE<sub>45-319</sub>, CPE<sub>53-319</sub>, CPE<sub>69-319</sub>, CPE<sub>103-319</sub>, and CPE<sub>168-319</sub>) and a C-terminal CPE truncation fragment (CPE<sub>1-291+</sub>) have been expressed in *E. coli*. When these CPE fragments were tested for CPE-like cytotoxicity for Vero cells, CPE<sub>1-319</sub>, CPE<sub>37-319</sub>, and CPE<sub>45-319</sub> showed cytotoxic activity; this cytotoxicity was neutralizable by a monoclonal antibody which blocks the cytotoxicity of native CPE. However, no cytotoxic effects were induced by the remaining fragments. When CPE<sub>1-319</sub> and the truncation fragments were tested for their competitive binding abilities against <sup>125</sup>I-CPE, CPE<sub>1-319</sub> and all six N-terminal truncation fragments displayed similar binding activity as native CPE, while CPE<sub>1-291+</sub> showed disrupted binding. The ability of CPE<sub>1-319</sub> and the N-terminal truncation fragments to "insert", i.e., to remain associated with membranes following protease treatment, was also tested. Only the cytotoxic CPE fragments (CPE<sub>1-319</sub>, CPE<sub>37-319</sub>, and CPE<sub>45-319</sub>) displayed "insertion". The noncytotoxic fragments were all released from membranes. The cytotoxic CPE fragments were also the only fragments which were capable of forming large complex. Collectively, these results; i) suggest that the minimum size cytotoxic CPE fragment lies between CPE<sub>45-319</sub> and CPE<sub>53-319</sub>, ii) suggest the noncytotoxic N-terminal truncation fragments are blocked at the second step (referred to as "insertion") of CPE action, iii) are consistent with our previous studies which identified a receptor-binding region at the extreme C-terminus of CPE, iv) do not support the existence of a second receptor-binding region located elsewhere in CPE, and v) confirm that sequences in the N-terminal half of CPE are required for post-binding step(s) in CPE action.



## BIOLOGICAL EFFECTS AND VACCINE POTENTIAL OF STAPHYLOCOCCAL ENTEROTOXIN B MUTANTS: N23K AND F44S

<sup>1</sup>M. A. Woody, <sup>1</sup>T. Krakauer, <sup>2</sup>J. Bill, and <sup>1</sup>B. G. Stiles

<sup>1</sup>Toxinology Division, USAMRIID, Frederick, MD, U.S.A. and

<sup>2</sup>Supragen, Inc., Lakewood, CO, U.S.A.

The biological activities of two *Staphylococcus aureus* enterotoxin B (SEB) mutants, N23K and F44S, were studied in a lipopolysaccharide (LPS)-potentiated mouse model. Neither mutant was lethal when tested at 5 µg/mouse, a protein amount equivalent to ~15 LD<sub>50</sub> of SEB. The mutants did not elevate serum levels of tumor necrosis factor (TNF), a cytokine associated with toxic shock in animals, but there was a 30-fold increase in TNF serum levels in SEB-injected mice. Both mutants were serologically similar to SEB in an ELISA with polyclonal anti-SEB sera. Mice immunized with alum containing either N23K, F44S, or SEB developed comparable anti-SEB titers. When immunized animals were each challenged with 10 µg of SEB (~30 LD<sub>50</sub>), there was an 80% survival rate for mice immunized with either N23K or F44S. The survival rate among mice immunized with SEB was 87% whereas none of the naive animals and only 7% of those given alum alone survived the SEB challenge. These studies suggest that the N23K and F44S mutants of SEB are biologically less active than SEB, yet retain common epitopes useful for eliciting a protective antibody response.

## **CARRY OVER OF AFLATOXINS IN MILK AND MILK PRODUCTS IN PAKISTAN AND THEIR POSSIBLE CONTROL.**

**Mansoor A. Ahmad, Butool A. Khan,  
Zuzzer A. Shamsuddin And Mobeen A. Khan.**

**MYCOTOXINS LABORATORY  
PCSIR LABS. COMPLEX, KARACHI-75280, PAKISTAN.**

During the survey spread over ten years, 441 samples of milk were collected. Twenty one percent samples were found positive for aflatoxin  $M_1$  and the range being 0.03 to 0.98  $\mu\text{g/kg}$ . There are two hundred thousand buffaloes around Karachi. Twenty four buffaloes were given 6.75 mg of aflatoxin  $B_1$  each through feed per day. The secretion of  $AFM_1$  in milk was 5.92  $\mu\text{g/kg}$ . Thus total aflatoxin  $M_1$  secreted was 59.2  $\mu\text{g}$  on the basis of average of ten kg of milk given by each buffalo per day. The conversion ratio was 0.88% from  $AFB_1$  to  $AFM_1$ . The feed concentrate containing 1688  $\mu\text{g/kg}$  was detoxified on a pilot plant scale with ammonia and upto 97.1% detoxification was achieved. The detoxified feed was fed to 24 buffaloes and  $AFM_1$  could not be detected in majority of the buffaloes milk. Aflatoxin  $M_1$  was detected in 14% samples of imported powder milk with a range of 0.027 to 0.467  $\mu\text{g/kg}$  on basis of reconstituted milk. The total number of 1879 samples of feed ingredients used in buffaloes ration by local farmers were analyzed and the percentage of aflatoxins contamination was: cottonseed cake 65%, corn 49%, rapeseed cake 29%, wheat bran 37%, sorghum 24%, sunflower cake 66%, and stale bread 62%. Some of them has over 10 mg/kg of total aflatoxins.

FUNGAL CONTAMINATION OF MEDICINAL PLANT PRODUCTS  
AND AFLATOXINS DETECTION

I.Rizzo, G.Vedoya, E. Varsavsky and H. Frade  
INSTITUTO NACIONAL DE MEDICAMENTOS  
Av.Caseros 2161 (1264) Capital Federal  
ARGENTINA

Consumption of medicinal plant products for infusion has been increased in the last years. In concordance special interest exist to ensure their quality control. Their soil origin, their bad manipulation and drying (12% of humidity content) allow the presence of high contamination of bacteria and fungi.

The object of this study was: A) to investigate the fungal contamination B) to identify the fungal species C) to determine their toxicogenic capacity and D) to select the best detection method for aflatoxin in pure and mixed products, working with TLC methods and monoclonal Ab.

The results showed: a total fungi plate count between  $10^2$  to  $10^6$  cfu/g, that were reduced in 2 log orders after infusion (in boil water, 5 min.). The predominant fungi were: *Aspergillus* (100%), *Penicillium* (70%), *Alternaria* (40%), *Cladosporium* (25%). The *Aspergillus* spp found were: *A.niger* (28.32), *A.flavus* (22.38), *A.ochraceus* y *versicolor* (15%), *A.nidulans* (10.4) y *A.glaucus* (8.9). The 55.5% of the strains of *A.flavus* resulted toxicogenic using the rice as substrate.

The Ab monoclonal method for the Aflatoxin determination in pure and mixed products was the best compared with TLC method due to the clean-up procedure was very labourious.

METABOLIC BASIS OF THE PROTECTIVE EFFECT OF  
SOME FOOD INGREDIENTS AGAINST DEOXY-  
NIVALENOL TOXICITY

L.V.Kravchenko, O.L.Oboljsky, L.P.Zacharova  
and V.A.Tutelyan

Institute of Nutrition, Moscow 109801, Russia

Because it is probably not possible to eliminate completely exposure of humans to mycotoxins, it is important to find out the ways of reducing of toxicity of the toxins entered into the organism. Our investigation have shown that same food ingredients wich arewidely used in medical practice modify metabolism and toxicity of trichothecene mycotoxin deoxynivalenol (DON). The addition of w 3 fatty acids to the diet markedly increased fecal excretion of DON glucuronides and had a smaller effect on the DOM-1 excretion. The supplementation of diet with selenium was accompanied by drastic increase of formation and elimination of DON and DOM-1 glucuronides both in urine and feces. No effect of feeding lignin was seen on the toxicity and metabolism of DON. Pectin supplementation of diet increased the elimination of DON glucuronides but did not influence significantly DOM-1 excretion. There was a correlation between glucuronides excretion and increase in activities of microsomal UDP-glucuronosyltransferases in the liver of rats fed diets with fish oil, selenium or pectin. Lignin-supplemented diet had no effect on xenobiotic-metabolizing enzymes in the liver and small intestine. These results show that increase of formation of glucuronides appears to be an essential factor to explain the protective effects of same food ingredients against DON toxicity.

MACROCYCLIC TRICHOTECENES IN ARGENTINIAN *BACCHARIS*  
*CORIDIFOLIA* AND *ARTHEMISIOIDES*

I. Rizzo, E. Varsavsky and H. Frade  
INSTITUTO NACIONAL DE MEDICAMENTOS  
Av. Caseros 2161 (1264) Capital Federal  
ARGENTINA

Toxic disease produced by shrubs of *B. coridifolia* and *B. arthemisioides* to livestock is very common in our country.

Toxicity of *B. coridifolia* (37 plants of both sexes) and *B. arthemisioides* (18 plants) from different regions of Argentina was investigated for our group. Toxicogenic capacity of 11 isolates of endophytes from the same plants of *B. coridifolia* (Cabral, et. al; 1989) was determined. Extraction methods of Jarvis (1985, 1987) for plants and fungi with TLC detection was used. Results were: A) *B. coridifolia*: 100% plants were positive for roridin A (RA) and RE and verrucarins A (VA) and VJ; 16.2% for RD and 2.7% for RH. No differences in behaviour was shown among female and male plants. B) *B. arthemisioides*: RA, RE and RD were present in higher concentrations than VA and VJ and all of them more concentrated than in *B. coridifolia*. C) Endophytes fungi: 45.5% of the isolates were toxicogenic for the same roridins and verrucarins but in very low concentrations. They showed variable and confuse behaviour according to the production media used.

This is the first reference of macrocyclic trichotecene in *B. arthemisioides* and the first record of *B. coridifolia* macrocyclic in Argentina.

## TOXICOKINETICS OF TRITIATED DIHYDROMICROCYSTIN-LR IN SWINE

<sup>1</sup>R.R. Stotts, <sup>2</sup>R.A. Twardock, <sup>2</sup>G.D. Koritz, <sup>2</sup>W.M. Haschek,

<sup>2</sup>R.K. Manuel, <sup>2</sup>W.L. Hollis, and <sup>2</sup>V.R. Beasley

<sup>1</sup>U.S. Army Medical Institute of Chemical Defense, Aberdeen  
Proving Ground, MD 21010-5425 U.S.A., <sup>2</sup>University of Illinois  
College of Veterinary Medicine, 2001 S. Lincoln Ave., Urbana IL  
61801 U.S.A.

The toxicokinetics of tritiated dihydromicrocystin-LR ( $[^3\text{H}]2\text{H-MCLR}$ ) were studied in anesthetized, specific pathogen free (SPF) pigs. Two doses of the radiolabeled plus nonlabeled 2H-MCLR were administered IV and one dose was given via an isolated ileal loop. The IV doses of 25  $\mu\text{g/kg}$  and 75  $\mu\text{g/kg}$  were rapidly removed from the blood. At either IV dose, more than half the radiolabel from  $[^3\text{H}]2\text{H-MCLR}$  present in the blood at one minute post-dosing was cleared by 6 minutes. The blood clearance at the 75  $\mu\text{g/kg}$  dose was slower than that at the 25  $\mu\text{g/kg}$  dose. Accordingly, at the high dose, the concentrations of the toxin in blood were disproportionately higher from 10 minutes after dosing until the study ended 4 hours later. The decreased clearance is presumably due to decreased elimination as a consequence of the hepatic injury that was observed histologically. Following administration of  $[^3\text{H}]2\text{H-MCLR}$  at 75  $\mu\text{g/kg}$  via the ileum, the peak concentration of toxin in blood was achieved at 90 minutes after dosing, when  $[^3\text{H}]2\text{H-MCLR}$  concentration in portal venous blood was 3.6 times higher than in peripheral venous blood. Although bile production varied, following IV dosing, radioactivity in bile from the gall bladder was detected as early as 12 minutes post-dosing in one animal. This study demonstrates the rapid removal of  $[^3\text{H}]2\text{H-MCLR}$  from the blood of anesthetized swine and the presence of the radiolabel in the bile within 30 minutes after dosing.

## DESIGN OF INHIBITORS FOR RICIN A-CHAIN

Xiangyang Chen, Todd Link, and Vern L. Schramm  
Department of Biochemistry, Albert Einstein College of Medicine  
Bronx, NY 10461, U.S.A.

Ricin A-chain (RTA) is a potent toxic protein which inactivates ribosomes by catalyzing the depurination of adenosine 4324 in 28S rRNA. The minimal substrate for recognition by ricin A-chain has been reported to be a RNA 10-mer featuring a stem-loop structure containing the GAGA tetraloop. The mechanism of action of RTA is similar to N-nucleoside hydrolases which have oxycarbonium character at the transition state. Inhibitors with oxycarbonium character at the depurination site for RTA were designed to incorporate the oxycarbonium transition state and stem-loop structure required for ricin A-chain action. Formycin A and phenyliminoribitol, known nucleoside hydrolase inhibitors, were incorporated into RNAs via chemical synthesis. Binding studies of RTA with these synthetic nucleotide and oligonucleotides were carried out by ultrafiltration or filter-binding methods. Formycin A 5'-monophosphate (FMP) showed <1 mM binding affinity to RTA, although FMP is known to bind at higher concentrations. Weak binding of RTA with tetramer GFGA was observed. Stem-loop 10-mer GCGGFGACGC also showed weak binding to RTA, with little improvement of affinity compared to tetramer GFGA. These inhibitors showed no protection against RTA in reticulocyte-lysate translation assays. Stem-loop RNAs featuring oxycarbonium character at the RTA target site are not efficient transition state analogue inhibitors of RTA.

Supported by research contract DAMD 17-93-C-3051 from the United States Army Medical Research and Materiel Command.

## FUMONISIN B1 ALTERS THYMUS FUNCTION IN MICE

Elena A.Martinova, Institute of Nutrition, 109240 Moscow, Russia

Fumonisin B1(FB1) is classed as a *Fusarium* mycotoxin produced by some related species and is known as a toxic agent and carcinogen for some animals and human. Immune action of FB1 can be carried out either via direct interaction between FB1 and lymphocyte surface receptors or via biological activity of sphingolipids. FB1 has been shown to alter immune function and sphingolipid metabolism in BALB/c mice (E.A Martinova & A.H.Merrill, Jr, 1995, *Mycopathologia*) and modulate both a content of sphingomyelin cycle products and CD3-antigen (Ag) expression in immune cells (Martinova E.A. et al, 1995, *Biochemistry* (Moscow)).

Mouse thymus is most sensitive to FB1 exposure *in vivo* and *in vitro*. CD3-(common T lymphocyte Ag), CD4-(helper/inductor), CD8-(cytotoxic/suppressor) and CD45-(membrane-associated phosphotyrosine phosphatase prevents CD2 and TCR/CD3 receptor complex from generating intracellular signals by dephosphorylating some phosphoproteins involved in the signal transduction) antigen expression on thymus cells has been shown to be disrupted for 2hr after FB1 exposure. CD45-Ag has reserved high level of its expression during 4 days. D-Sphingosine displays the significant effect on CD-Ag expression but different compared with FB1. The CD-Ag expression alteration by FB1 causes a disruption of immune response to T-dependent antigens *in vivo*: the level of antibody -(Ab) (or plaque-) forming cell (PFC) decreases after single FB1 i.p. administration in dose-dependent manner. FB1 alters the B cell function in the thymus: causes increasing the PFC to T-dependent Ag in mouse thymus. The small number of B cells in the thymus play a role of Ag-presenting cells (APC) in the negative selection of thymocytes, and they are not designed to secrete the Ab to foreign Ag. Change-over from APC to PFC may be significant for B thymic cells in the clonal deletion of the CD4+CD8- thymocytes. Low doses of FB1, as well as D-sphingosine, lead to the apoptotic changes in the thymocytes for 6-12hr after i.p. administration. Dietary consumption of FB1 contaminated corn can lead to immune deficiency connected with T cell function.



## SENSITIVITY OF ALPHA AMANITIN TO OXIDATION BY A SYSTEM OF LACTOPEROXIDASE AND HYDROGEN PEROXIDE

<sup>1</sup>A. M. Zheleva, <sup>2</sup>Z. D. Zhelev, <sup>3</sup>D. Michelot and <sup>4</sup>N. E. Lazarov

<sup>1</sup>Department of Chemistry and Biochemistry, <sup>2</sup>Department of Molecular Biology and Immunology, <sup>4</sup>Departement of Anatomy, Histology and Embriology, Medical University, 6000 Stara Zagora, Bulgaria, <sup>3</sup>Laboratoire de Chimie, Museum National d'Histoire Naturelle, U.R.A. 401 du CNRS 63 rue Buffon 75231 Paris CEDEX 05 France

The cytotoxin alpha-amanitin is a bicyclic octapeptide occurring in high concentration in the deadly poisonous mushroom *Amanita phalloides*.

Possibility alpha amanitin to be oxidized by a system containing lactoperoxidase and hydrogen peroxide was investigated. By UV spectroscopy an obvious decrease in the absorption intensity of the toxin UV spectrum at 305 nm was found after 24 hours incubation with the enzyme and hydrogen peroxide in 20 mmol phosphate buffer pH 7.5. Formation of a new product was detected by TLC analysis. It was also established a reduced *in vivo* toxicity of the so treated toxin. Considerable decrease in the structural changes induced in the hepatocytes of mice injected with alpha amanitin treated by lactoperoxidase and hydrogen peroxide comparing to those of mice treated only with the native toxin were found. Probable formation of an alpha amanitin oxidized product has been discussed.

INCORPORATION OF A 35 kDa PURIFIED PROTEIN FROM  
Loxosceles VENOM TRANSFORMS HUMAN ERYTHROCYTES  
INTO ACTIVATORS OF AUTOLOGOUS COMPLEMENT  
ALTERNATIVE PATHWAY.

D.V. Tambourgi, F.C. Magnoli, V.R.D. Von Eickstedt, \*Z.C. Benedetti,  
V.L. Petricevich, and W. Dias da Silva. Instituto Butantan and

\*Univ. de São Paulo, São Paulo, S.P., Brazil.

Inoculation of Loxosceles venoms produces local necrosis occasionally accompanied by systemic intravascular clotting and hemolysis. We have analyzed *in vitro* the role of C system on the lysis of human erythrocytes ( $E^h$ ) pretreated with Loxosceles venoms.  $E^h$  were treated with whole venom of *L. laeta*, *L. gaucho* or *L. intermedia* or with purified proteins from these venoms and incubated with C-sufficient (Cs-NHS) or C9-depleted autologous (C9d-NHS) serum. Hemolysis was determined spectrophotometrically and deposition or removal of C components was analyzed by FACS.  $E^h$  exposed to venoms or to a purified 35 kDa protein from *L. intermedia* were lysed by Cs-NHS but not by C9d-NHS. The lysis was blocked by heating the serum at 52°C or  $Ca^{++}/Mg^{++}$  chelation by EDTA, but not by  $Ca^{++}$  chelation with EGTA. Deposition of C1q, C2, factor B, C4, C3 and C5 on the venom-treated  $E^h$  cell surface occur during C activation, but the autologous C regulatory proteins DAF and CD59 were not removed. Conversion of C-resistant  $E^h$  into C-susceptible  $E^h$  by the venom is accompanied by incorporation of a 35 kDa venom protein on their cell surfaces. The data suggest that C system plays an important mediator role in lysis of erythrocytes and, by extension, other cell types able to incorporate on their cell surfaces the lytic promoting factor of Loxosceles venoms.

# A COMPARATIVE BIOCHEMICAL STUDY OF COMPONENTS WITH LETHAL AND DERMONECROTIC ACTIVITIES FROM THE VENOM OF SPIDERS OF THE GENUS *LOXOSCELES*

K. C. Barbaro<sup>1</sup>, L. Morhy<sup>2</sup>, M. V. Sousa<sup>2</sup>, V. R. D. Eickstedt<sup>1</sup> and I. Mota<sup>1</sup>

<sup>1</sup>Instituto Butantan - 05503-900 - S. Paulo - SP and <sup>2</sup>Centro Brasileiro de Sequenciamento de Proteínas - Universidade de Brasília - 70910-900 - Brasília - DF - BRAZIL

Toxins (32-35 kDa) were isolated from venoms of *Loxosceles gaucho*, *L. laeta* and *L. intermedia* by SDS-PAGE followed by blotting to PVDF membrane. The blotted samples were sequenced in an ABI 477A/120A automatic protein sequencer. *L. gaucho* toxin and *L. intermedia* toxin showed many identical residues in their N-terminal sequence. However, *L. laeta* toxin showed a lower homology. A search against the NR databank at NCBI showed high levels of identity between *L. gaucho* (60%) and *L. intermedia* (75%) toxins and *L. reclusa* (North American brown spider) toxin. We also detected 85% of similarity between *L. gaucho* and *L. intermedia* toxins and *L. reclusa* toxin. Preliminary results suggested an unexpected identity (47%) and similarity (68%) between the *L. laeta* toxin and astacin (crayfish small-molecule proteinase). These toxins were also submitted to capillary electrophoresis peptide mapping in a 270A-HT CE System (Applied Biosystems) after *in situ* partial hydrolysis of the blotted samples with HCl 3N at 80°C during a week. The results obtained suggested that *L. intermedia* protein is more homologous with *L. laeta* toxin than *L. gaucho* toxin and revealed a smaller homology between *L. intermedia* and *L. gaucho*. Altogether these findings suggested that toxins from *Loxosceles* species are probably homologous toxins. Amino acid analysis is under way for further confirmation and quantitation of this homology.

Support: Instituto Butantan, FAPESP (92/1926-6) PADCT, FUB-UnB.

REGULATION OF STORAGE PROTEIN PRODUCTION IN  
ENVENOMATED HOST LARVAE PARASITIZED BY  
*EUPLECTRUS* SPP. (HYMENOPTERA: EULOPHIDAE)

<sup>1</sup>Sandra L. Brandt, <sup>1</sup>Thomas A. Coudron, <sup>2</sup>Doug Jones and <sup>3</sup>Abdur Raquib

<sup>1</sup>USDA-ARS-Biological Control of Insects Research Laboratory, 1503 S.  
Providence, Research Park, Columbia, MO 65203-3535 USA, <sup>2</sup>Graduate  
Center for Toxicology, Univ. of Kentucky, 204 Funkhouser Bldg.,  
Lexington, KY 40506-0054 USA, <sup>3</sup>Dept. of Entomology, Univ. of Missouri,  
1-87 Agric. Bldg., Columbia, MO 65211 USA

The venom of the ectoparasitoids *Euplectrus comstockii* and *Euplectrus plathypenae* cause alterations in the titer of endogenous proteins found in the hemolymph of their hosts. In early larval stadiums of acceptable hosts (hosts that support the complete development of the parasitoids) envenomation stimulates the premature production of late larval storage proteins. This demonstrates the ability of the venom to cause the production of a gene product that normally occurs at a different growth stage of the host. In contrast, envenomation of the last larval stadium results in a decrease in the titer of storage proteins at a growth stage when these proteins are normally present in abundance. Therefore the venom appears to cause bidirectional alterations of the storage protein titers within acceptable hosts. These alterations in the titer of storage proteins were found to occur in isolated tissues that were separated from the intact endocrine or central nervous systems of the host. The likely mechanisms of action of the venom is to regulate the transcription and/or translation of the gene(s) that encode for the storage proteins. In nonacceptable hosts (hosts that do not support the complete development of the parasitoids) envenomation did not cause as pronounced alterations in the titer of endogenous hemolymph proteins. This suggests nonacceptable hosts are somewhat refractory to the mechanism(s) of action of the venom.

CLONING AND EXPRESSION OF TOXIN Cn 5 FROM THE SCORPION  
*Centruroides noxius* HOFFMANN.

<sup>1</sup>C. Garcia, <sup>2</sup>E. S. Calderon-Aranda, <sup>1</sup>F. Bolivar, <sup>1</sup>L.D. Possani and  
<sup>1</sup>B. Becerril Instituto de Biotecnologia, Universidad Nacional  
Autonoma de Mexico, Avenida Universidad, 2001, Apartado Postal  
510-3, Cuernavaca, MEXICO 62271, <sup>2</sup>Centro de Biotecnologia,  
Universidad Autonoma Estado Morelos, Cuernavaca, MEXICO 62271.

Due to our interest in understanding the structure-function relationship of the Na<sup>+</sup>-channel blocking peptides from scorpion venoms, we have cloned and expressed toxin Cn 5 from the Mexican scorpion *Centruroides noxius* Hoffmann. The amino acid sequence was determined and the complete gene (Cngt II) for this toxin was also obtained from a cDNA library. For the expression the gene was edited, using PCR, the signal peptide and the last two lysines were removed because they are not present in the mature peptide. Furthermore, a methionine was added at the amino terminal in order to separate the recombinant toxin from the fusion protein with maltose binding protein (MBP) by means of CNBr cleavage. For purification and expression we have used a kit (New England Biolabs) that provides a cytoplasmic vector (pMal-C) with the tac promoter, the malE gene (codes for the maltose binding protein) and a polylinker where we inserted the edited gene for toxin Cn 5. The vector also provides a recognition site for the factor Xa protease in order to cleave the protein of interest from MBP. Thus far, we have expressed a fusion peptide of approximately 50 Kd, this is the expected size since the MBP has 42 Kd and the Cn 5 is close to 7.5 Kd. After purification the fusion peptide was digested with factor Xa and two peptides of the expected size were obtained. The identity of the Cn 5 recombinant toxin was confirmed by three independent lines of evidence: i) amino acid determination of the first 20 amino acid residues of the recombinant peptide, ii) nucleotide sequence and, iii) by using polyclonal antibodies against native Cn 5 by western blotting. We obtained a high yield (10 % of total protein) of hybrid protein, however, the yield of the free recombinant toxin was very low (1% of the hybrid) due to problems during refolding of the peptide.

Acknowledgments: Supported in part by grant Howard Hughes Medical Institute No. 75191-527104, DGAPA-National University of Mexico No. 205893 and Consejo Nacional de Ciencia y Tecnologia of Mexico.

## AMINO ACID SEQUENCE OF *Tityus discrepans* SCORPIONS TOXINS

<sup>1</sup>G. D'Suze, <sup>2</sup>F. Corona, <sup>1</sup>C. Sevcik and <sup>2</sup>L. Possani<sup>1</sup>.

Instituto Venezolano de Investigaciones Científicas, Centro de Biofísica y Bioquímica, Laboratory of Cellular Neuropharmacology, Apdo. 21827 Caracas 1020-A Venezuela. <sup>2</sup> Instituto de Biotecnología, Universidad Nacional Autónoma de México Apdo. 510-3, Cuernavaca 62271 México.

*Tityus discrepans* is the most common producer of life threatening scorpionism in Venezuela. The soluble proteins in its venom were fractioned using a Sephadex® G-50 column (0.9 x 200 cm, 18.9 ml/h/cm<sup>2</sup>), equilibrated and eluted with 20 mM ammonium acetate buffer at 25°C, pH 4.7. The peptides were purified chromatographing twice on reverse phase high performance liquid chromatography (HPLC) through Protein C4 (Vydac) columns. Toxins were reduced, carboxymethylated and digested. The digested peptides were separated with HPLC and sequenced by automatic Edman degradation. Primary structures of two toxins, which we call Td1 and Td2 were determined. The homology of Td2 with  $\gamma$  toxin from *T. serrulatus* is higher than with the latter and Td1. The homology of the Td1 and  $\gamma$  toxin is higher at their N-terminals. The biological activities of the toxins were studied on the sartorius neuromuscular preparation of the frog (*Hyla crepitans*). The action of Td1 is similar to toxin  $\gamma$  from *T. serrulatus* in muscarinic effects, in the action on membrane potential and, on the acetylcholine release. Td2 induces pancreatitis like toxin  $\gamma$ , but lacks the muscarinic effect of  $\gamma$  toxin. More information about molecular polymorphism of scorpion toxins should help understanding the evolutionary relationships among scorpions, and on relationships between toxin structure and mechanism of action. [Partly funded by CONICIT (Venezuela) grant S1-2086 (CS) and by grant 75191-527104 from the H. Hughes Medical Institute and from CONACyT (México)].

## ANTIGENIC CHARACTERIZATION OF TOXIN 2 FROM DE VENOM OF THE SCORPION *Centruroides noxius* HOFFMANN

G. Gurrola-Briones, A. Méndez-Teapila and L.D. Possani  
Department of Molecular Recognition and Structural Biology,  
Instituto de Biotecnología - Universidad Nacional Autónoma de  
Mexico, Avenida Universidad, 2001, Apartado Postal 510-3,  
Cuernavaca, MEXICO 62271.

Six different monoclonal antibodies (mAb) were produced against *C. noxius* toxin 2 (Cn2), one of the most potent toxins from the Mexican scorpions. These mAb define four distinct epitopes in toxin Cn2. The hybridomas named BCF-2 and BCF-3 are capable of producing mAb that neutralize *in vivo* the effect of 7.5 LD<sub>50</sub> doses of Cn2, assayed in mice (Zamudio et al., *Eur.J.Biochem.*, 204: 281-292, 1992). Samples of native toxin Cn2 were hydrolyzed with enzymes (*Staphylococcus aureus* V8, trypsin and Aparitic-N endopeptidase) and the corresponding peptides were separated by HPLC and assayed using an ELISA format, in order to access recognition of the various HPLC-peptides by the mAb, hence identifying the possible antigenic determinants of toxin Cn2. Digestion with protease V8 produced a fragment (dimeric peptide) corresponding to the amino acid sequences from position 3 to 15 and 54 to 66 (here included the disulfide bridge Cys12-Cys65), which was toxic to mice at the doses assayed (2 µg/20 g mouse weight). This same fragment of Cn2 is recognized by BCF-2 and BCF-3, which seems to indicate the participation of the N-terminal and C-terminal segments of toxin 2 in the toxic effect observed in mice. These findings also suggest that this might explain the *in vivo* neutralizing capacity of both mAb BCF-2 and BCF-3.

**Acknowledgements:** Supported in part by grant Howard Hughes Medical Institute No.75191-527104, DGAPA-National University of Mexico No. 205893 and Consejo Nacional de Ciencia y Tecnología of Mexico.

ISOLATION AND CHARACTERIZATION OF INSECT-SPECIFIC  
PEPTIDE TOXINS FROM THE VENOM OF THE SOUTH INDIAN  
RED SCORPION, *BUTHUS TAMILUS*

Wudayagiri Rajendra<sup>1</sup>, Haim Moscovitz<sup>2</sup>, Rafael Hermann<sup>2</sup>,  
Prabhakar V. Choudary<sup>2</sup> and Bruce D. Hammock<sup>2</sup>

<sup>1</sup>Department of Zoology, Division of Molecular Biology,  
Sri Venkateswara University, Tirupati - 517 502, A.P., India

<sup>2</sup>Department of Entomology and Environmental Toxicology,  
University of California, Davis, CA.95616-8584, USA

Recombinant nuclear polyhedrosis viruses (NPVs) expressing insect selective toxins, hormones or enzymes could enhance the insecticidal properties of the NPVs. Insect specific peptide neurotoxins have been isolated from the venom gland of South Indian Red Scorpion, *Buthus tamulus*. The purification of these toxins was performed by CM-cellulose ion exchange chromatography and HPLC. The degree of purity of the toxins was assessed by capillary electrophoresis and isoelectric focussing. CM-fractions of *B. tamulus* do not share common epitopes with other known insect selective neurotoxins AaIT, Lqh IT2, Lqh  $\alpha$ T and p tox as revealed by immune cross reactivity analysis with the antibodies of known scorpion toxins. The toxins (BtIT1 & BtIT2) induced flaccid paralysis characteristic to depressant neurotoxins in *Heliothis virescens* larvae but were non-toxic to *Sarcophaga falcipalpa* (blow-fly) larvae and mice. The ecological objective of this study is to construct recombinant baculovirus transfer vectors carrying BtIT1 and BtIT2 genes and to provide the basis for novel strategies in the development of new biopesticides.

<sup>1</sup>Address for correspondence



ISOLATION OF A PURIFIED TOXIN (MARTOXIN, MTX) FROM  
THE VENOM OF THE SCORPION BUTHUS MARTENSI KARSCH  
WITH ADRENERGIC AND NANC ACTIONS

GONG JIANPING<sup>1</sup>, R. MANJUNATHA KINI<sup>2</sup>, M.C.E. GWEE<sup>3</sup>, and P.  
GOPALAKRISHNAKONE<sup>1</sup>

Venom and Toxin Research Group, Departments of Anatomy<sup>1</sup> and  
Pharmacology<sup>3</sup>, Faculty of Medicine, and Bioscience Centre<sup>2</sup>, Faculty of  
Science, National University of Singapore, Lower Kent Ridge Road,  
Singapore 0511

A novel toxin was purified to homogeneity from the crude venom of the  
scorpion *Buthus martensi* Karsch (MKV) by a combination of gel filtration on  
a Bio-gel P-30 Column, and cation exchange chromatography on a POROS  
HS column, followed by reversed-phase chromatography on a R 2/H 4.6/50  
column on a BioCad perfusion chromatography workstation. We have named  
the toxin MARTOXIN (MTX). The effects of the toxin (MTX) on adrenergic  
and non-adrenergic non-cholinergic (NANC) responses were investigated  
using the rat isolated anococcygeus muscle (Acm) mounted in Krebs solution  
(37°C, 5%CO<sub>2</sub> in O<sub>2</sub>). MTX(10µg/ml) produced marked and sustained  
contractions of the Acm which were blocked by phentolamine (5µM).  
MTX(10µg/ml) also markedly relaxed the carbachol precontracted Acm; the  
relaxation was inhibited by N<sup>G</sup>-nitro-L-arginine-methylester (L-NAME)  
(50µM). Thus, MTX is a novel toxin which can mediate adrenergic as well  
as NANC responses in the rat Acm.

Supported by research grants (RP940304, RP870357 and RP940325) from NUS.

PURIFICATION, CHARACTERIZATION AND GENOMIC  
ORGANIZATION OF KTX<sub>2</sub> FROM *ANDROCTONUS*  
*AUSTRALIS*, A NEW INHIBITOR OF VOLTAGE AND CALCIUM  
ACTIVATED K<sup>+</sup> CHANNEL

Laraba-Djebari, F., Legros, C., Crest, M<sup>#</sup>., Céard, B., Romi, R.,  
Mansuelle, P., Jacquet, G<sup>#</sup>., Van Rietchoten, J., Gola, M<sup>#</sup>.,  
Rochat, H., Bougis P.E. and  
Martin-Eauclaire, M-F

From the Laboratoire de Biochimie, Ingénierie des Protéines, Unité de Recherche Associée 1455 du Centre National de la Recherche Scientifique, Faculté de Médecine Nord, Boulevard Pierre Dramard, 13916, Marseille, Cedex 20, France # and from the Laboratoire de Neurobiologie, Centre National de la Recherche Scientifique, 31, Chemin Joseph Aiguier, 13402, Marseille, Cedex 20. France.

Kalimotoxin has been originally described as an inhibitor of the intermediate conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel. Recent experiments using radioiodinated KTX show that Dendrotoxin (DTX) was able to displace the specific KTX binding to rat brain synaptosomes. Since DTX is a specific voltage-dependent K<sup>+</sup> channel blocker, this result suggests that KTX bind also to voltage-sensitive K<sup>+</sup> channel. We describe here the purification and the chemical and biological characterizations of a new kalimotoxin-like peptide, extracted from the *Androctonus australis* scorpion venom. KTX<sub>2</sub>-AaH shares 76% sequence homology with KTX. The main differences between their two sequences concern the 12 first N-terminal amino acid residues and two amino acid residues located in the region involved in the channel recognition. These differences may explain the decrease by fivefold of the KTX<sub>2</sub>-AaH blockage potency (K<sub>i</sub> = 110 nM) and channel binding affinity (K<sub>i</sub> = 50 pM).

Using degenerated primers, a 370 bp cDNA encoding the KTX<sub>2</sub>-AaH precursor has been amplified by PCR technique from a venom gland cDNA library of *Androctonus australis*, revealing that the KTX<sub>2</sub>-AaH precursor was organized like those of scorpion toxins active on Na<sup>+</sup> channel.

## $\alpha$ -LATROTOXIN CHANGES THE PHOSPHORYLATION OF SYNAPTOSOMAL PROTEIN P65.

N.H.Himmelmreich, L.G.Storchak and N.G.Pozdnyakova.

A.V.Palladin Institute of Biochemistry, Ukrainian Academy of Sciences, Kiev 252030, Leontovich Str. 9, Ukraine.

A presynaptic neurotoxin  $\alpha$ -latrotoxin (LTX) is known as an active protein component of the black widow spider venom. This toxin is a unique tool induces the process of neurotransmitter release in a calcium-free medium and without mobilization calcium from intracellular stores. Our recent data show that under LTX action vesicular transmitter, but not the cytoplasmic is released. In the present study we have investigated the effect of LTX on protein phosphorylation using intact synaptosomes isolated from rat brain. By studying the  $^{32}\text{P}$ -labelled proteins following the incubation of  $^{32}\text{P}$ -prelabelled synaptosomes in the presence of LTX in calcium-free or calcium-supplemented bathing solution, we have found that in nominally calcium-free medium LTX increases the labelling of protein P65. This increase is more appreciable when exposure with the toxin is for 30s than for 10s and is equal nearly 150% of steady-state phosphorylation. And vice versa exposure of  $^{32}\text{P}$ -prelabelled synaptosomes in the presence of LTX in calcium medium leads to a reduction in the phosphorylation of protein P65. After exposure for 10s this reduction is slight and equal 5-15%, but after exposure for 30s level of P65 phosphorylation decreases to 70-60% of steady-state phosphorylation. We hypothesize that the cycle of phosphorylation/dephosphorylation of synaptosomal protein P65 is important for inducing the exocytotic process by LTX.

POTENTIAL-DEPENDENCE OF INTERACTION OF  $\alpha$ -LATROTOXIN  
CHANNELS IN PLANAR LIPID MEMBRANE WITH ANTIBODIES  
TO  $\alpha$ -LATROTOXIN AND THE EFFECTS OF PRONASE

<sup>1</sup>A.N.Chanturiya, <sup>2</sup>O.Ya. Shatursky, <sup>2</sup>A.N.Nikolaenko and <sup>2</sup>V.K.Lishko

<sup>1</sup>LTPB/NICHD - NIH, Bethesda, MD 20892 U.S.A.,

<sup>2</sup>A.V.Palladin Institute of Biochemistry, Kiev 252030, Ukraine.

The major toxic component of Black Widow spider (*Latrodectus mactans tredecimguttatus*) venom,  $\alpha$ -latrotoxin, is known to form ionic channels in different membranes.  $\alpha$ -latrotoxin channels in planar lipid membrane were treated with antibodies to latrotoxin or with pronase added to different sides of the membrane. It was found that antibody addition to the same side as latrotoxin (*cis*) decreased channel conductance at positive potentials across the membrane. On the contrary, *trans* side addition of antibodies changed the channel conductance at both positive and negative potentials: at positive potential conductance first slightly increased then decreased more than 50%; at negative potential it decreased much faster to only about 20% of the initial value. No dependence on membrane potential was found for pronase treatment of incorporated channels. For both *cis* and *trans* application of pronase channel selectivity for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ba}^{2+}$  and  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Li}^+$  ions did not change significantly but  $\text{Cd}^{2+}$  block was decreased. *Trans* pronase treatment also resulted in some rectification of I-V curves and an increase of channel conductance. We interpret these findings as evidence that channel position and/or conformation in the membrane depend on membrane potential.

CLINICAL ASPECTS AND TREATMENT OF SCORPION STINGS IN  
THE REGION OF RECIFE - BRAZIL

<sup>1</sup>M.C. Guarnieri, <sup>1</sup>P.K. Lima, <sup>1</sup>D.C.C. Pimentel, <sup>1</sup>K.C. Maia, <sup>1</sup>L.R. Andrade,  
<sup>1</sup>R.C. Valença, <sup>1</sup>C.M.L., Vasconcelos, <sup>2</sup>M.L. Porto and <sup>2</sup>A.E. Oliveira Jr.

<sup>1</sup>Depto. de Biofísica e Radiobiologia - Universidade Federal de Pernambuco -  
Recife - Brasil, <sup>2</sup>CEATOX - Hospital da Restauração - Recife - Brasil.

Envenomation by scorpions occurred predominantly in the metropolitan area of Recife, reflecting the high population density of this region and bad sanitary conditions of some leaving places. The aim of this study is to present these accidents (361 cases), recorded over a period of April 1992 to December 1994, attended at Restauração Hospital - Toxicological Accompany Center. All cases where the animal was brought to the Hospital were identified as *Tityus stigmurus*. The majority of the victims received healthy care until six hours after the accident (72.9%). At the site of the bite the signs and symptoms most commonly observed were pain (78.1%), paresthesia (16.6%), edema (15.7%), cutaneous lesion (11.3%) and hyperemia (6%). The main general symptoms including vomiting (10.2%), sudoresis (7.5%) and sleepiness (6.3%). The treatment used in 76.7% of the cases were restrict to intravenous analgesic plus local infiltration with lidocain. The serotherapy treatment was carried out only in 7.2% of the victims, being all children. The present work concluded that although frequent, these accidents are not serious agreeing to the envenoming by *Tityus stigmurus* described in others Northeast regions of Brazil.

Supported by: CNPq-PROPESQ, FACEPE

# NEUROTRANSMITTER SECRETION STIMULATED BY $\alpha$ -LTX ACTIVATES PHOSPHORYLATION OF PKC SUBSTRATES IN PRIMARY NEURONES.

Delio Mercanti<sup>§</sup>, Maria Teresa Ciotti<sup>§</sup>, Marta Hribal<sup>§</sup>, Luisa Milazzo<sup>§</sup>,  
Patrizia Casalbore<sup>#</sup> & Alfonso Grasso<sup>#</sup>.

CNR, <sup>§</sup>Institute of Neurobiology and <sup>#</sup>Institute of Cell Biology, V.le  
Marx 15/43, 00137 ROMA - ITALY. Tel. 039 (6) 8292592 - Fax  
039 (6) 86090370.

Stimulation of cerebellar granule cells with  $\alpha$ -latrotoxin ( $\alpha$ LTX) results in a substantial increase in glutamatergic transmitters secretion and in a transient altered  $[Ca]_i$ . The molecular mechanism(s) leading to the neurotransmitter release is still unclear. An ionotropic-like action as well as the activation of a signal transduction pathway have been suggested to operate in the toxin-stimulated neurosecretion. Indeed the recently identified neuron-specific  $\alpha$ LTX receptor could indicate a possible activation of the secretory process which originates at the cell surface where the engagement of the receptors initiates a cascade of events in which some post-translational protein modification(s) are likely to occur. Here we report on the phosphorylation(s) kinetics induced by acute treatment of the  $^{32}P$  labelled neurones with nanomolar concentration of  $\alpha$ LTX. The radiolabelled cellular lysates were analyzed by two dimensional gel electrophoresis and the maps obtained were compared and the corresponding single spots quantified. Changes in protein phosphorylation were observed after treatment of the cells with  $\alpha$ LTX. In particular a progressive  $^{32}P$  incorporation was observed in three proteins previously identified as PKC specific substrates (MARCKS, GAP43, pp43). Specific kinase inhibitors like Bisindolylmaleimide (BMM) and Staurosporine practically abolished the  $\alpha$ LTX effect. Our results suggest an implication of the PKC and/or its substrates in the signal transduction events triggered by  $\alpha$ LTX binding to specific presynaptic receptors.

## $\alpha$ -LATROTOXIN-INDUCED FUSION OF NEGATIVELY CHARGED LIPOSOMES.

Ya.T.Terletskaya and I.O.Trikash.

Palladin Institute of Biochemistry, Ukrainian Academy of Sciences, Kiev, Ukraine.

$\alpha$ --Latrotoxin (LTX) is a major toxic component of the venom of black widow spider *Latrodectus mactans tredecimguttatus*. This toxin is a protein of approximately 130 kD with an isoelectric points of 5.2 and has a powerful presynaptic activity. It is known, that the toxic action of LTX is due to both formation of cation channels through the plasma membrane and triggering neurotransmitter secretion. LTX stimulates exocytosis, i.e. the process whereby synaptic vesicles fuse with the presynaptic membrane. In the present research we investigated the effect of LTX on fusion of large vesicles, which better approximate biological membranes. Membrane intermixing was monitored with the fusion assay based on resonance energy transfer. The  $Tb^{3+}$ -DPA fusion assay was used for monitoring intermixing of vesicle contents. In this study was demonstrated that LTX promotes the fusion of negatively charged liposomes and this effect is enhanced by calcium ions. The rate and efficiency of liposome fusion increased with lowering pH. Using the fluorescent probe ANS the hydrophobicity of LTX molecule as a function of pH was studied. It was found that lowering pH is resulted in the exposition of hydrophobic domains. It has been suggested that LTX can undergo conformational changes which make it possible to insert into the lipid bilayer. The information regarding the region of neurotoxin responsible for its anchorage into lipid bilayer is discussed.

# MICROASSAYS FOR THE INVESTIGATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN SMALL-SCALE VENOM SAMPLES.

P. Escoubas, T. Nakajima

Suntory Institute for Bioorganic Research, Mishima-Gun, Shimamoto-Cho,  
Wakayamadai, Osaka 618, Japan.

Successful investigation of active components in venoms is often hampered by limiting amounts of biological material. Toxicity and pharmacological assays consume relatively large amounts of material and bioassay-guided isolation of minor components is often impossible, with classical techniques. Conversely, the increased sophistication of analytical instrumentation allows for the structural study of minute amounts of material.

We have therefore devised new micro-scale assays for the study of neurotoxic components in arthropod venoms.

The first technique is a micro-injection assay using *Drosophila melanogaster* adults. Flies are injected with volumes up to 50 nl, using glass micropipettes. To calibrate the method, a comparative toxicity study of 7 scorpion venoms was undertaken, using a standard cricket bioassay as a reference. Results show differences in toxicity against the two insect species and a net gain (up to 100 times) in amounts of material utilized.

The second technique is an insect smooth-muscle assay, using a 100  $\mu$ l bath chamber. The effects of several neurotransmitters, 7 scorpion venoms and selected ion-channel antagonists were studied to determine the pharmacological profile of the preparation.

Results demonstrate that the muscle response is selective and that differential activity with the toxicity assays make it a useful tool in the isolation of active components from unknown venoms.



## A CASE OF ENVENOMING BY PORTUGUESE MAN-O'WAR FROM THE BRAZILIAN COAST.

Freitas, J.C.<sup>1</sup>, Schiozer, W.A.<sup>2</sup> and Malpezzi, E.L.A.<sup>1</sup>

1. Biosciences Institute and Marine Biology Center, University of São Paulo, São Paulo, SP, Brazil. 2. University Hospital of University of São Paulo, São Paulo, SP, Brazil.

The most common marine envenoming are inflicted by cnidarians, and the skin is the primary organ affected. Cnidarians have cnidocyte cells bearing organelles called nematocysts, and they inject a mixture of powerful toxins just by skin contact. There are several cases of jellyfish envenoming, and in some cases Portuguese man o'war stings can be serious or even lethal. Here we report an individual case on envenoming caused by the cnidarian Portuguese man o'war. The reported local reactions and the clinical symptoms are similar to those already known for *Physalia* species. A 40-year-old healthy male (S.C.C.N.) was swimming off the Enseada beach, Guarujá, south coast of São Paulo, on 02.28.1994 when he was stung on the left hand indicator finger. He removed the tentacles using the fingers of the right-hand. Erythema and edema appeared few minutes after contact, followed within days, by ulceration, pigmentary changes, local muscle contractures and beginning of tissue necrosis. Few days later the patient purulent finger was submitted to surgical debridement with isolation of necrotic tissue. As the necrosis reached the extensor tendon of the finger the patient exhibited an important functional sequel with impairment of mobility of the interphalangean and metacarpo-phalangean articulation. Now, he is making physiotherapy exercises, trying to recover his finger movements. As far as we know it is the first clinical case documented for the Brazilian shores. Supported by FAPESP, CNPq.

## THE GILLS OF FISH AND CRUSTACEANS: VULNERABILITY AND RESISTANCE TO CYTOLYTIC TOXINS

<sup>1</sup>D.Mebs, <sup>2</sup>C.Giese and <sup>2</sup>B.Werding

<sup>1</sup>Zentrum der Rechtsmedizin, University of Frankfurt, <sup>2</sup>Institut für Allgemeine und Spezielle Zoologie, University of Giessen, Fed.Rep.of Germany.

When exposed to compounds like gramicidin, saponin and to cytotoxins from sea anemones, gills of marine as well as of fresh water fish undergo considerable morphological changes due to the cytolytic action of these toxins. The gill filaments are damaged by disruption of the epithelial cells and of the structure of the secondary lamellae. Although fish like the anemonefish (*Amphiprion* species) may develop some resistance to the sea anemone toxins, their gill-epithelium is still sensitive to other membrane-active compounds. On the other hand, the gills of crustaceans are highly resistant to the membrane-damaging action of these compounds. This is obviously due to the fact that the chitin cuticle covering the gills is an effective barrier protecting the cells from the direct exposure to the cytolytic agents.

**TRIMETHYL SULFONIUM CHLORIDE ISOLATED FROM MIDGUT GLAND OF *Aplysia brasiliiana* PREVENTS THE VAGUS NERVE EFFECTS ON THE TOAD'S HEART.**

Freitas, J.C.1, Freitas, A.2 & Roque, N.F.2

1. Biosciences Institute and Marine Biology Center, University of São Paulo, São Paulo, Brazil.

2. Chemistry Institute, University of São Paulo, São Paulo, Brazil.

In a previous study, a methanol extract obtained from the midgut gland of the sea hare *Aplysia brasiliiana*, induced contraction in invertebrate cholinceptive muscles and in other experiments it prevented the toad's heart diastolic arrest when the vagus nerve was stimulated (Freitas, J.C., 1977, Comp.Biochem.Physiol., 56C:57-61). In this work the aqueous part of the methanol extract of the midgut glands obtained from 35 specimens of *A. brasiliiana* was precipitated by ammonium reinecke salt at pH 9.8. After exchange of reinecke ion by chloride ion, the material was submitted to a cellulose column chromatography.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra and elementary analysis of the active fractions identified the compounds as choline chloride, tetramethyl ammonium chloride and trimethyl sulfonium chloride ( $[\text{C}_3\text{H}_9\text{S}]^+\text{Cl}^-$ ). The pharmacological assays using the *in situ* toad's vagal-heart preparation allowed to test each of the above compound and it was demonstrated that  $[\text{C}_3\text{H}_9\text{S}]^+\text{Cl}^-$  is responsible for the prevention of the toad's heart diastolic arrest induced by electrically stimulated vagus nerve.

Supported by FAPESP, CNPq, Brazil

# EXPRESSION OF THE GENE ENCODING THE $\beta$ -SUBUNIT OF STONUSTOXIN FROM STONEFISH, *SYNANCEJA* *HORRIDA*.

Ghadessy, F. G., Jeyaseelan, K., Khoo, H.E. and Yuen, R.

Department of Biochemistry, Faculty of Medicine and the Bioscience Centre,  
National University of Singapore, Kent Ridge Crescent, Singapore 0511.

We have previously reported the purification of stonustoxin from the venom of the stonefish, *Synanceja horrida*. It is a heterodimeric protein ( $\alpha$  and  $\beta$  subunits) and displays haemolytic, myotoxic, oedema-inducing, hypotensive and neurotoxic activities. The toxin's precise mechanism(s) of action is, however, not known.

The gene encoding the  $\beta$ -subunit of stonustoxin has been cloned from a venom gland cDNA library and characterized by DNA sequencing. The predicted amino acid sequence showed no homology with other toxins or lethal factors reported in the main protein sequence databases suggesting that it may be a novel multi-class toxin. In this report we present the results of expression studies leading to the production of this novel protein in *Escherichia coli*.

## MORPHOLOGICAL CHANGES CAUSED BY STONUSTOXIN IN SKELETAL AND SMOOTH MUSCLE

Low, K.S.Y.; Gopalakrishnakone, P.\*; Gwee, M.C.E.; Yuen, R.+ and Khoo, H.E.+.

Venom and Toxin Research Group, Departments of Pharmacology, Anatomy\* and Biochemistry+, Faculty of Medicine, National University of Singapore.

Stonustoxin (SNTX), a peptide toxin isolated from the venom of the stonefish, *Synanceja horrida*, has previously been shown to cause contractures in both skeletal and smooth muscle. In the present study, isolated chick biventer cervicis muscles (CBCM) and strips of rat aorta were exposed to SNTX 10  $\mu\text{g/ml}$  for 30 minutes, and the tissues were then fixed and processed for examination under a transmission electron microscope. Examination of the CBCM revealed areas where muscle fibres showed extensive degeneration and disorganization, with enlarged and disrupted mitochondria. These fibres were often found in close association with guard cells and other muscle fibres which were normal in appearance. Nerve terminals appeared to be unaffected by SNTX, with no clear depletion of synaptic vesicles when compared to control tissues. Examination of blood vessels found in the CBCM showed the endothelial cells to be separated from the smooth muscle cells by large gaps; however, these smooth muscle cells, as well as those in the rat aorta, were apparently identical to those in control tissues. From the observations, it appears that SNTX (10  $\mu\text{g/ml}$ ) causes extensive damage of skeletal muscle without producing any apparent morphological changes in smooth muscle.

INHIBITION OF MEMBRANE-BOUND ACETYLCHOLINESTERASE  
BY A NEW AChE INHIBITOR FROM THE ZOANTHID  
*PARAZOANTHUS AXINELLAE*

<sup>1</sup>J. Strupi-Šuput, <sup>1</sup>L. Kamarič, <sup>2</sup>T. Turk, and <sup>1</sup>D. Šuput

<sup>1</sup>School of Medicine, Institute of Pathophysiology, EDT Lab, P.O.Box 11,  
SLO-61105 Ljubljana, and <sup>2</sup> Department of Biology, BFT, Ljubljana

It has been shown recently that the extract from the zoanthid crust coral *Parazoanthus axinellae* is lethal to crabs and mice. The *in vivo* effects of the extract were typical of an AChE inhibition. The purified substance isolated from the extract inhibits the purified AChE from electric eel in 1  $\mu$ M concentration, which is comparable to the potency of phystiostigmin, the first described natural AChE inhibitor. Since the effects and possible therapeutic applications of this new substance would depend on the specificity of actions on different membrane-bound AChEs it seemed reasonable to study the effects of the purified inhibitor on BuChE in human plasma and on AChE in bovine superior cervical ganglion, iris, and on human red blood cells.

Each sample was homogenized and the activity of AChE was measured by continuous pH-metric titration at constant substrate concentrations, pH 7.4, and 25 °C. In a control experiment the inhibition by ethanolic extract of the purified electric eel AChE was measured using the same method.

The maximal inhibition of the human erythrocyte AChE was about 80%. The same concentration inhibited only 25% of BuChE activity in plasma. Although the difference was significant it is not sufficient to consider the new inhibitor as a selective AChE inhibitor. The new inhibitor has a structure unseen among the cholinesterase inhibitors before. Therefore it may be an interesting starting point to develop new drugs to be used in human disease. Preliminary experiments on the insect AChE suggest that the inhibitor could also serve as a model for development of new pesticides.

ShK is a potassium channel blocker from the Caribbean sea anemone *Stichodactyla helianthus*

<sup>1</sup>O. Castañeda, <sup>2</sup>E.G. Rowan, <sup>3</sup>L. Young, <sup>2</sup>A.J. Harvey and <sup>2</sup>E. Karlsson  
(<sup>1</sup>Facultad de Biología, Universidad de La Habana, Cuba, <sup>2</sup>Strathclyde  
Institute for Drug Research, 204 George Street, Glasgow, U.K.

<sup>3</sup>Department of Biochemistry, Biomedical Center, Box 576, 751 23  
Uppsala, Sweden)

Since many years ago sea anemones constitute a source of neuroactive compounds mainly related to modulate kinetic aspects of sodium channels. In addition, it has been discovered potassium channel blocking activity in the aqueous extract of *Stichodactyla helianthus* and *Bunodosoma granulifera* (Karlsson *et al.* 1991) and recently two potassium channel blockers have been isolated: PqK (Azeiros *et al.* 1993) and ShK (Castañeda *et al.* 1995).

ShK is a 35 amino acid residues peptide, three disulphide bonds and 1054 Da. The toxin is a pre-synaptic facilitator as demonstrated by its action upon neuromuscular transmission in chick biventer cervicis neuromuscular preparations indirectly stimulated. It also inhibit the binding of dendrotoxin (a probe for voltage-dependent potassium channels) to rat brain synaptosomal membranes, i.e. the correspondents  $IC_{50}$  and  $K_i$  values as well as the type of inhibition, were determined. The toxin also diminishes the potassium channel currents in rat dorsal root ganglion neurons and in NG108-15 neurons in culture.

## CARDIOVASCULAR EFFECTS OF EQUINATOXIN III

R. Frangež, and D. Šuput

Laboratory for Cell Physiology and Toxinology, Institute of Pathophysiology,  
University of Ljubljana, School of Medicine, 61105 Ljubljana, Slovenia

Equinatoxin III is one of the three isotoxins isolated from the Adriatic sea anemone *Actinia equina*. Its effects *in vivo* and on the isolated organs have not been studied, yet. Cardiorespiratory arrest due to the coronary vasoconstriction is the main reason for the lethality of a similar equinatoxin II. Equinatoxin III is more haemolytic but less toxic than equinatoxin II. The aim of our study was to compare mechanisms responsible for the lethal activity of the two toxins.

The toxin was tested *in vivo* on rats and *in vitro* on isolated pig coronary arteries. Equinatoxin III was injected intravenously and the arterial blood pressure, the electrocardiogram, and respiratory activity were measured. The tension of the vascular smooth muscle *in vitro* was measured isometrically by a mechanoelectrical transducer.

*In vivo* the effects of equinatoxin III were similar to the effects of equinatoxin II. Immediately after application of the toxin the animal stopped breathing, and soon a marked arrhythmia developed. Analysis of the blood samples revealed haemolysis, elevated plasma potassium level and degranulation of neutrophils and basophils. Artificial respiration and vagotomy had no effect on the survival of the experimental animal. Intravenous application of potassium chloride solution had no significant effects. Not equinatoxin III in 1.5  $\mu$ M concentration, nor potassium (15 mM final concentration) in the bath had any effects on the contraction of the pig coronary artery.

We conclude that hyperkalemia can contribute to the lethality of the toxin but is not its primary cause. It also seems that the drop of coronary perfusion observed on the Langendorff's guinea pig hearts is not a result of a direct action of equinatoxin III. On the other hand, we can not exclude direct action of the toxin on smaller blood vessels, eg. arterioles. The effects of substances released from granulocytes still remains to be evaluated.



**CYTOTOXIC AND NEUROTOXIC EFFECTS INDUCED BY HALITOXIN ISOLATED FROM *Amphimedon viridis* (PORIFERA).**

Freitas, J.C.<sup>1,2</sup>; Malpezzi, E.L.A.<sup>1,2</sup>; Costa, L.V.<sup>1,2</sup>; Berlinck, R.G.S.<sup>3,2</sup>; Almeida, A.M.P.<sup>3</sup>; Ogawa, C.A.<sup>3</sup>; Sanchez, M.A.A.<sup>3</sup>; Hajdu, E.M.<sup>4</sup>. 1.Biosciences Institute, University of São Paulo, São Paulo, Brazil. 2.Marine Biology Center, University of São Paulo, São Sebastião, Brazil. 3.Chemistry Institute of São Carlos, University of São Paulo, São Carlos, Brazil. 4.Institut voor Systematiek en Populatiebiologie, University of Amsterdam, Amsterdam, Netherlands.

Halitoxin was previously isolated from the sponge *Amphimedon compressa* (= *Haliclona rubens*) from the Pacific Ocean. Lately this toxin was identified in *Amphimedon viridis*, and it was partially characterized (Schimitz, F.J. *et al.*, J.Org.Chem., 43: 3916, 1978). In this work we isolated and characterized the halitoxin from *A. viridis* and biological activities were studied. Sponge samples were collected on the north coast of São Paulo State. After extraction with CH<sub>2</sub>Cl<sub>2</sub>/MeOH 1:1 and evaporation of the volatile solvents, the aqueous suspension was partitioned with n-hexane, ethyl acetate and CHCl<sub>3</sub>/MeOH. The H<sub>2</sub>O/MeOH and CHCl<sub>3</sub>/MeOH fractions showed potent hemolytic activity (EC<sub>50</sub> ± SEM = 3.13 ± 0.52 and 0.73 ± 0.09 µg/ml, respectively). The purification of the fraction CHCl<sub>3</sub>/MeOH resulted the isolation of halitoxin, identical by RMN-<sup>1</sup>H and by RMN-<sup>13</sup>C, COSY <sup>1</sup>H-<sup>1</sup>H and HETCOR, to the previously isolated from *A. compressa*. Halitoxin, in lower concentrations (0.01 to 1.0 µg/ml) showed antimitotic effect (EC<sub>50</sub> ± SEM = 2.78 ± 0.17 µg/ml) on sea urchin eggs; and in higher concentrations (10 and 100 µg/ml) cellular lysis occurred. Potent hemolytic action (EC<sub>50</sub> ± SEM = 2.2 ± 0.5 ng/ml) was also verified in 0.5 % erythrocyte suspension. Sucrose-gap assays using the isolated crab leg nerve show a progressive decrease in the evoked spikes, simultaneously with a slow membrane depolarization with successive additions of 5 µg up to 15 µg/100 µl bath. A partial recover was obtained on washing. Acute toxicity by i.v. injection in mice showed lethal action with 1.4 mg/Kg in 40 min. These preliminary pharmacological data indicates that the action may be unspecific on biological membranes, opening pores or altering the structure of the bilayer affecting the normal cell membrane processes. Supported by: FAPESP, CAPES and CNPq.

## 4-AMINOPYRIDINE ANTAGONIZES THE LETHAL EFFECTS OF SAXITOXIN (STX) AND TETRODOTOXIN (TTX)

Benton, B.J., D.L. Spriggs, B.R. Capacio, and F.-C.T. Chang  
Pathophysiology Division, USAMRICD, APG-EA, MD 21010, USA.

The effectiveness of 4-aminopyridine (4-AP) in reversing STX and TTX-induced lethal effects was investigated in unanesthetized guinea pigs chronically instrumented for the recordings of electrocorticogram (ECoG), diaphragmatic electromyogram (DEMG), neck muscle electromyogram (NEMG) and Lead II ECG. The animals received a lethal dose of either STX or TTX (5  $\mu$ g/kg; im) which resulted in a progressive cardiorespiratory depression characterized by bradycardia, bradypnea and a reduction in DEMG and ECoG amplitudes. Positive pressure intratracheal ventilation was administered at the first sign of diaphragmatic failure ( $\approx$ 10 min post-toxin). Therapy, which consisted of 4-AP (2 mg/kg; im), epinephrine (0.1 mg/kg, im) and 6-8 ml of 7.5% sodium bicarbonate (ip; to reduce the severity of toxin-induced acidemia), was administered within 2 min after artificial ventilation. The effect of 4-AP was striking in that within 30 min, the toxin-induced functional blockade of the diaphragm, bradypnea, bradycardia and depressed cortical activity could all be reversed and gradually returned to a level comparable to that of the control. The animals were typically able to breathe spontaneously within 1 hr following 4-AP. In conclusion, results from this study showed that STX and TTX-induced cardiorespiratory failure can be reversed by 4-AP - a potassium channel blocker. The optimal therapeutic dose of 4-AP was determined to be 2 mg/kg. The extent to which 4-AP's therapeutic effectiveness can be further optimized by using other pharmacological adjuncts is currently under investigation.

## **Isolation and Identification of a Key Metabolite from 3,4-Diaminopyridine Administration to Guinea Pigs.**

Robert L. von Tersch, Benedict R. Capacio, Christopher E. Byers and Fat-Chun T. Chang

Pharmacology and Pathophysiology Divisions, United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010

Tetrodotoxin (TTX) and saxitoxin (STX) are potent sodium channel antagonists that have been shown to produce cardiorespiratory depression in guinea pigs. Administration of aminopyridines, such as 4-amino- or 3,4-diaminopyridine, has been utilized to ameliorate the STX- and TTX-induced effects. Pharmacokinetic studies of 3,4-diaminopyridine have demonstrated the appearance of a major metabolite in guinea pig plasma approximately 15-30 minutes after drug administration. The compound has a slightly shorter retention time than the parent 3,4-diaminopyridine in the high performance liquid chromatographic (HPLC) assay used. Current isolation procedures involve microfiltration of the plasma followed by HPLC analysis of the filtrate. Nuclear magnetic resonance spectroscopy and gas chromatography/mass spectrometry of the metabolite will be discussed with respect to the toxicology of amino aromatics.

# 11-OXO TETRODOTOXIN AND A SPECIFICALLY LABELLED <sup>3</sup>H-TTX

B.Q.Wu<sup>1</sup>, C.Y.Kao<sup>1</sup>, M.Yotsu<sup>2</sup>, T.Yasumoto<sup>2</sup>, and  
S.R.Levinson<sup>3</sup>

<sup>1</sup>SUNY Downstate Med Ctr, Brooklyn, NY 11203,

<sup>2</sup>Tohoku University, Sendai, Japan,

<sup>3</sup>Univ. of Colorado Med Ctr, Denver, CO 80262.

Currently used ligands for the voltage-gated sodium channel have shortcomings. A commercially available <sup>3</sup>H-saxitoxin has the <sup>3</sup>H on the C-11 methylene C from which it can readily exchange with solvent H. A diffusely labelled <sup>3</sup>H-TTX with limited specific activity is not available commercially. Other non-commercial ligands are adducts of TTX, appreciably larger than TTX. We have made a stable specifically labelled <sup>3</sup>H-TTX of high specific activity by reduction of 11-oxo TTX (U.S. patent 5,288,870). This hydrated aldehyde of TTX was discovered as a natural analogue by Khora and Yasumoto (Tetrahedron Let. 30:349. 1989). Citrate-free TTX (Hebei) was oxidized by Fenton's reagent (FeSO<sub>4</sub>) or Pfitzner-Moffat reaction (dicyclohexylcarbodiimide, DMSO). 11-oxo TTX was isolated by HPLC, characterized by NMR and MS, and tested on the voltage-clamped frog skeletal muscle fiber. It blocked I<sub>Na</sub> specifically at ca. 3X the potency of TTX (Wu et al., Biophysical J. 59:261A, 1991). 11-oxo TTX was then reduced to TTX with sodium <sup>3</sup>H borohydride. The product required little work-up, had a specific activity of 3900 Ci/M (which could be increased), and bound specifically to the purified sodium channels of *Electrophorus electricus*. This marker could open new areas of studies on Na channels. (Supported by US Army contract DAMD17-87-C-7094 and NIH grant NS 14551)

## RECEPTOR BINDING ASSAY AND HPLC ANALYSIS OF BREVETOXINS IN ORGANISMS EXPOSED TO A FLORIDA RED TIDE

<sup>1</sup>T.A. Leighfield, <sup>2</sup>M.S. Henry, <sup>2</sup>R.H. Pierce, <sup>1</sup>F.M. Van Dolah, and <sup>2</sup>J. Zhou.

<sup>1</sup>Marine Biotoxins Program, Charleston Laboratory, U.S. National Marine Fisheries Service, Charleston, SC and <sup>2</sup> Mote Marine Laboratory, Sarasota, FL.

Suitability of a microplate receptor binding assay for the determination of brevetoxins in field samples was assessed using samples obtained during a persistent bloom of *Gymnodinium breve* on the west coast of Florida from September 1994 to January 1995. Oysters (*Crassostrea virginica*), clams (*Mercenaria mercenaria*), sea squirt (*Styela plicata*), and mullet (*Mugil cephalus*) were collected throughout the bloom at Mote Marine Lab, Bay Dock. Brevetoxins were extracted with acetone followed by a two-step column fractionation through silica gel then alumina. All samples were evaporated to dryness and brought up in methanol prior to analysis. The brevetoxin receptor binding assay was based on competition between [<sup>3</sup>H]PbTx-3 and PbTx-3 standards or unknown samples for binding to voltage dependent sodium channels in rat brain synaptosomes. Binding competition was carried out in a microplate format and quantified using a microplate scintillation counter to permit high sample throughput. Receptor assay results were compared with HPLC analyses on C18 columns, B&J OD-5 with 85/15 methanol/water (Mote) or Rainin Microsorb with 80/20 methanol/water (NMFS), at a flow rate of 1 mL/min with UV detection at 215 nm. *G. breve* cell counts at Bay Dock were >5000 cells/L beginning September 19, and were temporally variable with a maximum of 23,000,000 cells/L. Brevetoxin activity from oysters ranged from 0.05-0.38 µg PbTx-3 equivalents/g, with a maximum concentration observed during late October. Good correlation was observed between values obtained with the receptor assay and HPLC analyses. Sea squirts analyzed by receptor assay ranged from undetectable levels to 0.33 µg PbTx-3 equivalents/g. Detectable levels of brevetoxins were not observed in clams or mullet tissue.

COMPARATIVE RESPONSE OF HUMAN AND MURINE CELL LINES  
BY CELL BIOASSAY TO SODIUM CHANNEL ACTIVE MARINE  
TOXINS AND EXTRACTS

Ronald L. Manger, Linda S. Leja, Sue Y. Lee, James M. Hungerford, and  
Marleen M. Wekell

U.S. FDA, Seafood Products Research Center, Bothell, WA 98021

Cell-based bioassays offer a means for assessing potency of marine toxins without the restrictions and inconvenience of animal test methods. Our focus has been the development of cell-based methods for the detection of some of the more problematic marine toxins that act at the level of the voltage-gated sodium channel, inclusive of the ciguatoxins, brevetoxins, and saxitoxins. Based upon the response of mouse neuroblastoma cells *in vitro* this method has demonstrated high sensitivity and excellent correlation with animal toxicity studies, in particular the mouse bioassays currently used for monitoring. In the present study we have expanded upon our earlier investigations with mouse neuroblastoma cells by examining the application of human cell lines in this system. As anticipated, human cell lines derived from nerve tissue (neuroblastomas) were responsive in the cell bioassay system in a manner consistent with the presence of voltage-gated sodium channels, while specific dose dependent detection of these marine toxins was not observed with human cell lines derived from tissue lacking voltage-gated sodium channels (melanoma and colon carcinoma). Interestingly, the level of response and dependence upon assay conditions varied between the human neuroblastomas. Cell line dependent variations in the assay were also noted in comparisons of human and mouse neuroblastoma cell lines. These differences should be considered in attempts to model *in vivo* toxicity with *in vitro* assays. Recent studies have also demonstrated a high tolerance of matrix impurities and a wide acceptance of sample diluents by the cell assay. This has allowed significant simplification of sample preparation and has expanded the potential utility of these cell-based methods for the detection of marine toxins.

ASSAY OF SAXITOXIN IN SAMPLES FROM HUMAN VICTIMS OF  
PARALYTIC SHELLFISH POISONING BY BINDING COMPETITION  
TO SAXIPHILIN AND BLOCK OF SINGLE SODIUM CHANNELS.

<sup>1</sup>P. M. Bell, <sup>2</sup>B. D. Gessner, <sup>3</sup>S. Hall and <sup>1</sup>E. Moczydlowski

<sup>1</sup>Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, CT  
06520; <sup>2</sup>Sections of Epidemiology and Maternal, Child and Family Health,

Dept. of Health and Social Services, Anchorage, AK 99524;

<sup>3</sup>U.S. Food and Drug Administration, Washington, D.C. 20204.

The purpose of this study was to determine whether a saxiphilin-based assay can be used to measure relative concentrations of saxitoxin (STX) for applications in clinical toxicology. Saxiphilin is a soluble protein found in certain amphibians and reptiles that binds STX and various STX derivatives with high affinity and specificity. Saxiphilin from the bullfrog and garter snake bind [<sup>3</sup>H]STX with similar high affinity ( $K_D \approx 0.2$  nM), but exhibit differential affinity for neosaxitoxin. Serum and urine samples were collected from several human patients and victims of paralytic shellfish poisoning (PSP) that occurred in Kodiak, Alaska in May and June of 1994. Blind samples from these patients were assayed for the presence of STX or STX derivatives by a displacement competition assay using [<sup>3</sup>H]STX as the radioligand and frog or snake plasma as a source of saxiphilin. STX was used as the competitor ligand to generate standard ligand-displacement curves. Four urine samples were positive in the saxiphilin-based assay with effective STX concentrations ranging from 50 to 228 nM. One serum sample exhibited STX activity in the range of 9-27 nM. The most active urine specimen was also assayed on single rat muscle Na-channels incorporated into planar bilayers. This urine induced discrete blocking events with kinetic behavior characteristic of a mixture of native STX and a less toxic derivative of STX. The bilayer assay indicated an effective STX concentration of ~320 nM. In conclusion, a saxiphilin-based assay can be used for detection and quantitation of PSP toxins in clinical samples. (Supported by US Army Medical Research Institute and NIH.)

## ANALYSIS OF SAMPLES FROM A HUMAN PSP INTOXICATION EVENT USING A SAXITOXIN RECEPTOR ASSAY AND HPLC.

<sup>1</sup>G.J. Doucette, <sup>1</sup>M.M. Logan, <sup>1</sup>F.M. Van Dolah, and <sup>2</sup>S. Hall.

<sup>1</sup>Marine Biotoxins Program, National Marine Fisheries Service, Charleston, SC 29422-2607, <sup>2</sup>U.S. Food & Drug Administration, Washington, DC 20204.

Several cases of paralytic shellfish poisoning (PSP) were documented from Kodiak Is., AK in June, 1994. Serum and urine samples from six affected individuals, as well as AOAC extracts of the contaminated mussels were obtained through Alaskan state health officials. In four of the cases, serum samples were taken within 2 to 5 hours of consuming the shellfish. About 15 hours later, both serum and urine specimens were collected from three of these individuals. This sample set represented a unique opportunity to assess the kinetics of PSP toxin metabolism and clearance in humans. Human and shellfish samples, as well as cultures of toxic algae isolated from Kodiak Is. waters, were analyzed by saxitoxin receptor binding assay and/or HPLC. In all cases, the rank order of serum toxin levels measured by receptor assay corresponded well with the number of mussels consumed and the severity of symptoms presented. The highest serum toxin level obtained was 38 nM (1 h 55 min after eating over 20 mussels). No toxin was detected in any serum sample 20 h after ingesting the shellfish. However, toxin concentrations in the urine at this time ranged from 39 to 371 nM. This rapid clearance of toxin corresponds well with the time course of severe PSP symptomology. Total toxin concentrations determined by HPLC (as saxitoxin equivalents) differed from the receptor assay values by no more than 2-fold in 6 of 7 samples, including both serum and urine. The relative proportions of individual PSP toxins in cooked samples of the ingested shellfish were similar overall to those in the human fluids, with gonyautoxin 2 generally the most abundant (30-40 %). The sulfocarbamate derivatives C1 and C2 together comprised 50-70 % of the PSP toxins produced by three algal isolates examined, and about 35 % of toxins contained in the raw shellfish sample. After cooking, these two toxins accounted for less than 5 % of shellfish toxin, but interestingly represented up to 40 % of the toxins in certain human samples suggesting the possibility of biotransformations.



**AN OUTBREAK OF PARALYTIC SHELLFISH  
POISONING IN KODIAK,  
ALASKA: COMPARISON OF TOXIN DETECTION  
METHODS**

<sup>1</sup>S. Hall, <sup>2</sup>B. Gessner, <sup>3</sup>M. Poli,  
<sup>1</sup>P. Eilers, <sup>1</sup>S. Conrad, <sup>1</sup>E. Waldron, and <sup>1</sup>V. Brewer  
<sup>1</sup>Office of Seafood, U.S. Food and Drug Administration HFS-426,  
Washington, D.C. 20204 U.S.A., <sup>2</sup>State of Alaska, <sup>3</sup>Toxinology  
Division, Fort Detrick, Frederick, MD 21702-5011 U.S.A.

In the summer of 1994 there was a serious outbreak of paralytic shellfish poisoning (PSP) in Kodiak, Alaska. Fortunately, prompt medical intervention saved several lives that would otherwise have been lost. Shellfish, cultured dinoflagellates, and serum and urine from several surviving victims were assayed for the saxitoxins using a variety of methods. These studies provided both a comparison of some new and extremely sensitive methods for toxin detection, and a glimpse of the dynamics of the saxitoxins in human victims.

**INVESTIGATIONS OF *ALEXANDRIUM SP.* ISOLATES FROM  
KODIAK, ALASKA FOLLOWING AN OUTBREAK OF  
PARALYTIC SHELLFISH POISONING**

<sup>1</sup>E. Waldron, <sup>2</sup>B. Himelbloom, <sup>1</sup>S. Hall, <sup>1</sup>P. Eilers, and <sup>1</sup>S. Conrad  
<sup>1</sup>Office of Seafood, U.S. Food and Drug Administration HFS-426,  
Washington, D.C. 20204 U.S.A. <sup>2</sup>Fisheries Industrial Technology  
Center, University of Alaska, Fairbanks.

Dinoflagellates of the genus *Alexandrium* are generally recognized as the source of the saxitoxins, the compounds responsible for paralytic shellfish poisoning (PSP), in Alaskan waters, isolations of *Alexandrium* sp. were made from plankton samples taken near Kodiak, Alaska, in August 1994, following a series of PSP outbreaks there. These isolates were cultured and characterized using various parameters including toxin profile, growth rate, and chain formation.

FIELD PHYTOPLANKTON OBSERVATIONS  
AND MARINE BIOTOXIN MANAGEMENT

<sup>1</sup>S. Conrad, <sup>2</sup>G. Langlois, <sup>1</sup>P. Eilers, and <sup>1</sup>S. Hall

<sup>1</sup>Office of Seafood, U. S. Food and Drug Administration HFS-426,  
Washington, D. C. 20204 U. S. A., <sup>2</sup>California ~~State~~ Department of  
Health and ~~Human~~ Services, Berkeley CA

The successful management of seafood toxicity, to avoid human illness and severe economic impacts, requires a monitoring program to ensure that toxic seafood is not consumed and, preferably, is never harvested. Monitoring for toxicity is relatively expensive and can only detect toxicity once it is already there. Monitoring for toxic plankton provides some lead time, but has some of the same limitations as toxicity monitoring if the samples are preserved, shipped, and examined at a central laboratory. In contrast, plankton observations conducted in the field on live samples are much more efficient and provide an immediate view of the potential for toxicity in the days or weeks ahead. Techniques and equipment for conducting these observations are simple and effective. We are exploring the possibility of using field plankton observations to focus toxicity monitoring on the times and locations that are of greatest concern. To develop the required network of observers, including volunteers and shellfish growers, we have begun to produce training and reference materials. These include videotapes, printed manuals of color pictures, and preserved plankton samples in sealed capillaries. It is hoped that these materials will help us recruit and train field observers, and ultimately to improve the cost-effectiveness of marine biotoxin management.

## DOMOIC ACID ANALYSIS

P. Eilers, S. Conrad, and S. Hall

Office of Seafood, U. S. Food and Drug Administration HFS-426,  
Washington, D. C. 20204 U. S. A.

Domoic acid is a potent neurotoxin that can accumulate in seafood. Of all the known seafood toxins it is the most easily detected, since it has a strong UV chromophore. Routine analyses are conducted with simple isocratic HPLC and UV detection. During our production of large quantities of domoic acid for toxicological studies and reference standards we have encountered two difficulties: instability of concentrated aqueous solutions of domoic acid and resolution of tryptophan. Both problems have been noted elsewhere. The instability appears to be due to oxidative degradation at the conjugated double bonds in the longer side chain, and is greatly attenuated through the addition of an antioxidant. The resolution of domoic acid and tryptophan can be controlled through the modification of the mobile phase with triethylamine.

	<u>Abstract Page</u>	<u>Program Page</u>
Abbas, H.K.	89	20
Abd- Elsalam, M.A.	149	35
Abercrombie, J.	122	29
Adams, N.	181	40
Ahmad, F.	172	38
Ahmad, M.A.	185	41
Akramov, M.	132	32
Al-Ahaidib, M.S.	149	35
Almeida, A.M.P.	216	47
Alvarez, C.	99	22
Anderson, S.G.	164, 171	37, 38
Andrade, L.R.	70, 175, 176, 178, 204	15, 39, 45
Antuch, W.	100	2, 3
Arora, N.	80	17
Ashcom, J.D	98	22
Assakura, M.T.	70	15
Audebert, F.	179	39
Aurelian, L.	91	21
Azhar, A.	172	38
Baden, D.G.	117	28
Badria, F.A.	89	20
Baker, B.J.	65	14
Bakry, N.	74	16
Barbaro, K.C.	94, 194	21, 43
Bartels, F.	75	16
Bdolah, A.	157	36
Beasley, V.R.	189	41
Becerril, B.	106, 114, 196	25, 26, 43
Bell, P.M.	222	48
Benedetti, Z.C.	193	43
Benton, B.J.	217	48
Béress, L.	127	30
Bergdoll, M.S.	77	17
Berlinck, R.G.S.	216	47
Besada, V.	99	22
Bigalke, H.	75	16

	<u>Abstract Page</u>	<u>Program Page</u>
Biggs, D.	97	22
Bignami, G.	123	29
Bill, J.	184	40
Binscheck, T.	75	16
Bjarnason, J.B.	57	12
Blanco, L.	78	17
Blankemeyer, J.T.	134	32
Bloom, D.	91	21
Bolivar, F.	196	43
Bon, C.	50, 135, 179	11, 32, 39
Bonventre, P.	78	17
Bougis, P.E.	201	44
Boulain, J.-C.	62	13
Boyle, T.	79	17
Brandt, S.L.	195	43
Brazil, O.V.	146, 148	34, 35
Brewer, V.	224	49
Burkholder, J.M.	124	30
Burnett, J.W.	91	21
Burns, C.F.	134	32
Byers, C.E.	218	48
Byrnes, M.E.	92	21
Calderon-Aranda, E.S.	196	43
Capacio, B.R.	217, 218	48
Caplan, F.R.	122	29
Capuano, I.V.	108	25
Cardoso, D.F.	94	21
Carmichael, W.W.	90	20
Carsi-Gabrenas, J.M.	64	14
Casalbore, P.	205	45
Castaneda, O.	214	47
Céard, B.	201	44
Cestele, S.	110	26
Champagne, D.E.	104	24
Chang, F.-C.T.	217	48
Chang, I.M.	169	38
Chang, F.-C.T.	218	48
Chanturiya, A.N.	203	45

	<u>Abstract</u> <u>Page</u>	<u>Program</u> <u>Page</u>
Chapell, R.	51	11
Chavana, A.	165	37
Chavez, M.	100	23
Cheah, L.S.	111	26
Chen, X.	85, 190	19, 41
Chiappinelli, V.A.	63	14
Choi, E.	78	17
Choi, E.M.	169	38
Choudary, P.V.	199	44
Choumet, V.	50, 135, 179	11, 32, 39
Ciotti, M.T.	205	45
Cline, E.I.	97	22
Coffield, J.	74	16
Cogo, J.C.	70, 140, 141, 142	15, 33, 34
Colberg, T.R.	153	35
Conrad, S.	224, 225, 226, 227	49
Conti-Fine, B.M.	63	14
Corona, M.	114	26
Corona, F.	197	44
Costa-Cruz, J.W.M.	160	36
Costa, L.V.	216	47
Coudron, T.A.	103, 195	24, 43
Crest, M.	201	44
Crouch, R.C.	119	28
Cruz-Höfling, M.A.	70, 140, 142, 147	15, 33, 34
Cullen, C.	78	17
Cury, Y.	173, 174, 160	36, 38
D Abreu, A.C.F.	140	33
D'Suze, G.D.	96, 197	22, 44
Davis, S.E.	129	31
Day, P.	83	19
de Chastonay, J.	92	21
Delfin, J.	100	23
Delgado-Arias, J.A.	117	28
DelMar, E.	108	25
Delot, E.	50	11
Deshimaru, M.	55	12
Dias da Silva, W.D.	193	43

	<u>Abstract Page</u>	<u>Program Page</u>
Diaz, J.	100	23
Dickey, R.W.	117, 118, 119	28
Dodds, D.	53	11
Dolly, O.	60	13
Donato, J.L.	141	33
Dorandeu, F.	137, 151	33, 35
Doucette, G.J.	223	49
Ducancel, F.	62	13
Dunn, C.K.	108	25
Eickstedt, V.R.D.	194	43
Eilers, P.	224, 225, 226, 227	49
Eppler, K.G.	108	25
Ernst, S.	83	19
Escalona de Motta, G.	125, 126	30
Escoubas, P.	207	45
Fainter, L.K.	113	26
Fancheng Wang, F.	60	13
Farsky, S.H.P.	173, 174	38
Faure, G.	50	11
Ferreira, L.A.F.	158	36
Ferreira, M.L.	94	21
Finkelstein, R.A.	82	18
Fiordalisi, J.J.	63	14
Fletcher, P.L.	113	26
Fletcher, M.D.	113	26
Fletcher, J.E.	67, 143, 156	15, 34, 36
Fontana, M.D.	146, 148	34, 35
Fosbraey, P.	151	35
Fox, J.W.	57	12
Frade, H.	186	41
Frade, H.	188	41
Francis, B.	52	11
Franco, Y.O.	177	39
Franguez, R.	215	47
Freitas, J.C.	208, 210, 216	46, 47
Freitas, A.	210	46



	<u>Abstract Page</u>	<u>Program Page</u>
Fromen, C.	62	13
Fu, F.N.	76	16
Fukumaki, Y.	55	12
Fuller, F.H.	108	25
Garcia, C.	196	43
Gemski, P.	79	17
Gessner, B.D.	222, 224	48, 49
Ghadessy, F.G.	211	46
Giese, C.	209	46
Giglio, J.R.	141	33
Glasgow, Jr., H.B.	124	30
Gola, M.	201	4
Gonçalves, L.R.C.	173	38
Gong, Q.-H.	67	15
Gonzalez, Y.	100	23
González, I.	125	30
Gopalakrishnakone, P.	68, 111, 144, 152, 200, 212	15, 26, 34, 35, 44, 46
Gordon, D.	110	26
Gordon, V.M.	80	17
Goularte, F.C.	147	34
Granade, H.R.	117, 118, 119	28
Grant, G.A.	63	14
Grasso, A.	205	45
Guarnieri, M.C.	175, 176, 178, 204	39, 45
Gubensek, F.	54, 133	12, 32
Gurrola-Briones, G.	106, 198	25, 44
Gutiérrez, J.M.	66	14
Gwee, M.C.E.	111, 200, 212	26, 44, 46
Habermehl, G.G.	87, 158	20, 36
Habtemariam, S.	180	39
Hahn, B.S.	169	38
Hajdu, E.M.	216	47
Hall, S.	121, 222, 223, 224, 225, 226, 227	29, 48, 49

	<u>Abstract Page</u>	<u>Program Page</u>
Hammock, B.D.	199	44
Harvey, A.	59, 180	13, 39
Haschek, W.M.	189	41
Hattori, S.	55, 139	12, 33
Hawrot, E.	61	13
Heeg, H.	78	17
Heleno, M.G.	148	35
Helms, J.A.	53	11
Henry, M.S.	220	48
Hermann, R.	199	44
Hessinger, D.	93	21
Hill, R.	92	21
Himmelreich, N.H.	202	45
Himslbloom, B.	225	49
Hoffman, D.R.	107	25
Hollis, W.L.	189	41
Holmes, M.J.	130	31
Honkanen, R.E.	122	29
Hoover, D.	79	17
Horvat-Znidarsic, I.	135	32
Hribal, M.	205	45
Hubert, M.D.	156	36
Hungerford, J.	117	28
Hungerford, J.M.	221	48
Hyslop, S.	70	15
Imafuku, S.	91	21
Ismail, M.	149	35
Jacquet, G.	201	44
Jarvis, B.B.	88	20
Jerusalinsky, D.	180	39
Jett, M.	79	17
Jeyaseelan, K.	211	46
Jia, L.-G.	57	12
Jiang, M.-S.	143	34
Jiang, M.-S.	67	15

	<u>Abstract Page</u>	<u>Program Page</u>
Jiang, M.-S.	156	36
Jianping, G.	200	44
Johnson, J.	108	25
Johnson-Winegar, A.		17
Jones, D.	195	43
Jones, D.	102	24
Kaiser, I.I.	52	11
Kamaric, L.	213	47
Kao, C.Y.	219	48
Karlsson, E.	214	47
Kauffman, C.L.	91	21
Kazanov, E.	132	32
Kem, W.R.	92	21
Khafizon, A.	132	32
Khalifa, R.B.	110	26
Khamidov, D.Kh.	132	32
Khan, B.A.	185	41
Khan, M.A.	185	41
Khaytin, I.	92	21
Khoo, H.E.	211, 212	46
Kihara, H.	139	33
Kim, Y.S.	169	38
Kinamon, S.	157	36
King, T.P.	101	24
Kini, R.M.	56, 168, 200	14, 38, 44
Klimpel, K.R.	80	17
Knoepp, S.M.	129	31
Kochva, E.	157	36
Kokai- Kun, J.F.	183	40
Kordis, D.	54, 133	12, 32
Koritz, G.D.	189	41
Kornisiuk, E.	180	39
Krafte, D.S.	92	21
Krakauer, T.	184	40
Kral, R.M.	108	25
Krapcho, K.J.	108	25
Kravchenko, L.V.	187	41
Krishnamurthy, T.	95	21, 35

	<u>Abstract Page</u>	<u>Program Page</u>
Lallement, G.	137, 151	33, 35
Langlois, G.	226	49
Lanio, M.E.	99	22
Lanoue, B.A.	129	31
Lanzaro, G.C.	105	24
Laraba-Djebari, F.	201	44
Larionova, N.	100	23
Lazarov, N.E.	192	42
Lee, S.Y.	117, 221	28, 48
Legros, C.	201	44
Leighfield, T.A.	117, 220	28, 48
Leja, L.S.	221	48
Lélis, A.	159	36
Leppla, S.H.	80	17
Lerma, S.K.	165	37
Levinson, S.R.	219	48
Lewis, R.J.	116	28
Lima, P.K.	175, 176, 178, 204	39, 45
Link, T.	85, 190	19, 41
Lishko, V.K.	203	45
Logan, M.M.	223	49
Lomonte, B.	66	14
Long, S.R.	150	35
Low, K.S.Y.	212	46
Lownsohn, H.	181	40
Lu, S.-Y.	53	11
Lucas, S.M.	113	26
Mackessy, S.P.	58	14
Magnoli, F.C.	193	43
Mahnir, V.M.	92	21
Maia, K.C.	175, 176, 178, 204	39, 45
Maksymowych, A.B.	74	16
Malpezzi, E.L.A.	208, 216	46, 47
Manger, R.L.	117, 221	28, 48
Mansuelle, P.	201	44
Manuel, R.K.	189	41
Markland, F.S.	167, 170	37, 38

	<u>Abstract Page</u>	<u>Program Page</u>
Martin-Eauclaire, M-F	201	44
Martin, G.E.	119	28
Martinez, J.	100	23
Martínez, F.	106	25
Martinova, E.A.	191	41
Matev, I.B.	161	37
McClane, B.A.	73, 183	16, 40
McMorrow, S.	79	17
Mebs, D.	71, 209	15, 46
Melo, P.A.	134, 154	32, 36
Mereish, K.A.		19
Méndez-Teapila, A.	198	44
Méñez, A.	62	13
Meng, J.	52	11
Mercado, J.A.	125	30
Mercanti, D.	205	45
Michelot, D.	192	42
Milazzo, L.	205	45
Moczydlowski, E.	222	48
Moeller, P.D.R.	129	31
Monzingo, A.	83	19
Morera, V.	100	23
Morgan, T.K.	108	25
Morhy, L.	194	43
Morris, S.	91	21
Moscowitz, H.	199	44
Mota, I.	94, 194	21, 43
Musser, S.M.	117, 118, 119	28
Nagaoka, Y.	71	15
Nakagawa, K.	139	33
Nakajima, T.	207	45
Nakashima, K.	55	12
Narahashi, T.	115	27
Nicholson, G.M.	115, 138	27, 33
Nikolaenko, A.N.	203	45
Niu, L.H.	85	19
Nobuhisa, I.	55	12
Nomura, S.	139	33

	<u>Abstract Page</u>	<u>Program Page</u>
Nussenzveig, R.H.	104	24
O'Brien, A.D.	81	18
Oboljsky, O.L.	187	41
Oehme, F.W.		19, 20
Ogawa, T.	55, 139	12, 33
Ogawa, C.A.	216	47
Ohno, M.	55	12
Oliveira Jr., A.E.	175, 176, 178, 204	39, 45
Olson, M.	60	13
Omori-Satoh, T.	71	15
Onho, M.	139	33
Onoue, H.	139	33
Ownby, C.L.	69, 134, 143, 153, 154, 155, 164, 171	15, 32, 34, 35 36, 37, 38
Padron, G.	99, 100	22, 23
Parcej, D.	60	13
Parilis, I.I.	131, 132	32
Paronetto, C.C.	142	34
Patterson, G.M.L.	122	29
Pazos, F.	99	22
Pelhate, M.	110	26
Pennington, M.W.	92	21
Perez, J.C.	162, 163	37
Perin, M.S.	53	11
Pernot-Marino, I.	137, 151	33, 35
Petricevich, V.L.	193	43
Pierce, R.H.	220	48
Pierce, J.R.	165	37
Pimentel, D.C.C.	175, 176, 178, 204	39, 45
Pinto-Corrado, A.	141	33
Poli, M.A.	117, 228	28, 49
Ponraj, D.	152	35
Porto, M.L.	175, 176, 178, 204	39, 45
Possani, L.D.	106, 114, 196, 197, 198	25, 26, 43, 44

	<u>Abstract Page</u>	<u>Program Page</u>
Potter, L.T.	64	14
Pozdnyakova, N.G.	202	45
Prabhakaran, M.	95, 150	21, 35
Prado-Franceschi, J.	70, 141, 145, 159, 177	15, 33, 34, 36, 39
Purkerson, S.L.	64	14
Rajendra, W.	199	44
Ramos, L.A.	162	37
Ramos, M.	96	22
Raquib, A.	195	43
Reichl, A.P.	70	15
Reid, P.	60	13
Rein, K.S.	117, 128	28, 31
Ribeiro, J.M.C.	105	24
Ribeiro, J.M.	104	24
Rivara-Rentas, A.L.	125, 126	30
Riviére, G.	179	39
Rizzo, I.	186, 188	41
Robbe-Vincent, A.	50, 179	11, 39
Robertus, J.D.	83	19
Rochat, H.	110, 201	26, 44
Rodrigues-Simioni, L.	70, 140, 141, 142, 145, 147, 177	15, 33, 34, 39
Rodriguez, R.	100	23
Rodríguez, A.D.	126	30
Romi, R.	201	44
Roque, N.F.	210	46
Rosa, R.	126	30
Rosenberg, P.	51	11
Rosenthal, J.A.	61	13
Rowan, E.G.	214	47
Sabouraud, A.	179	39
Sakaki, Y.	55	12
Salikhov, R.S.	132	32

	<u>Abstract Page</u>	<u>Program Page</u>
Saliou, B.	50	11
Sanchez, M.A.A.	216	47
Sánchez, E.E.	163	37
Santaigo, M.P.	64	14
Santos, A.H.	105	24
Scherrmann, J.-M.	179	39
Scheuring, J.	182	40
Schiozer, W.A.	208	46
Schlimgen, A.K.	53	11
Schmidt, J.	60	13
Schramm, V.L.	85, 182, 190	19, 40, 41
Seebart, C.	52	11
Selistre de Araujo, H.S.	69, 143, 155	15, 34, 36
Servent, D.	62	13
Sevcik, C.	96, 197	22, 44
Shamsuddin, Z.A.	185	41
Sharma, S.K.	76	16
Shatursky, O.Y.	203	45
Shi, Q.-L.	61	13
Shier, W.T.	89	20
Shimohigashi, Y.	55	12
Shimokawa, K.-I.	57	12
Shoemaker, C.B.	105	24
Siddiqui, A.A.	105	24
Silva, W.	126	30
Simpson, L.L.	74	16
Singh, B.R.	76	16
Singh, Y.	80	17
Skelly, P.J.	105	24
Smith, L.	60	13
Sofer, S.	112	26
Sorkine, M.	179	39
Sousa de Silva, M.C.C.	173	38
Sousa, M.V.	194	43
Spriggs, D.L.	217	48
Stanilova, S.A.	161	37
Steighardt, J.	83	19
Stiles, B.G.	98, 184	22, 40
Storchak, L.G.	202	45
Stotts, R.R.	189	41



	<u>Abstract Page</u>	<u>Program Page</u>
Strauss, W.L.	64	14
Strupi-Suput, J.	213	47
Sueishi, K.	139	33
Sughra Ausat, F.S.	172	38
Suput, D.	135, 213, 215	32, 47
Svinth, M.	83	19
Tambourgi, D.V.	193	43
Tan, C.H.	144	34
Tattersall, J.E.H.	151	35
Teixeira, C.F.P.	160, 174	36, 38
Tejuca, M.	99	22
Terletskaya, Y.T.	206	45
Thwin, M.M.	144	34
Titus, R.G.	105	24
Todd, R.	109	25
Tomihara, Y.	166	37
Tosteson, T.	125	30
Trikash, I.O.	206	45
Tu, A.T.	65	14
Turk, T.	213	47
Tutelyan, V.A.	187	41
Twardock, R.A.	189	41
Ueno, E.	51	11
Urtizberea, M.	179	39
Vagin, A.N.	131	32
Vagina, O.N.	131	32
Valença, R.C.	175, 176, 178, 204	39, 45
Valentine, H.H.	64	14
Valenzuela, J.H.	104	24
Van Dolah, F.M.	117, 220, 223	28, 48, 49
Van Rietchoten, J.	201	44
VanWagenen, B.C.	108	25
Varsavasky, E.	186, 188	41
Vasconcelos, C.M.L.	175, 176, 178, 204	39, 45

	<u>Abstract Page</u>	<u>Program Page</u>
Vedoya, G.	186	41
Ver Lipps, B.	136	32
Vick, J.	181	40
von Bredow, J.	181	40
von Tersch, R.L.	218	48
Von Eickstedt, V.R.D.	193	43
Waldron, E.	224, 225	49
Wannemacher, R.W.		20
Weaver, W.R.	63	14
Wekell, M.M.	221	48
Welch, C.	79	17
Werding, B.	209	46
Wetherell, J.	151	35
White, S.P.	69	15
Wiebe, L.I.	97	22
Wilkins, T.D.	72	16
Wilson, H.I.	138	33
Winckler-Dietrich, V.	62	13
Wolowyk, M.W.	97	22
Woody, M.A.	184	17, 40
Wool, I.G.	84	19
Wright, J.	120	29
Wu, B.Q.	219	48
Yamakawa, Y.	71	15
Yang, K.Y.	169	38
Yasumoto, T.	219	48
Yonaha, K.	166	37
Yotsu, M.	219	48
Youle, R.J.	86	19
Young, L.	214	47
Yuen, R.	144, 211, 212	34, 46
Zacharova, L.P.	187	41
Zamunér, S.R.	145	34
Zappellini, A.	159	36

	<b><u>Abstract</u></b> <b><u>Page</u></b>	<b><u>Program</u></b> <b><u>Page</u></b>
Zaydenberg, I.	92	21
Zhand, R.	74	16
Zhelev, Z.D.	161, 192	37, 42
Zhelev, A.M.	192	42
Zhou, J.	220	48